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GIUSEPPE BASTIANELLI
(1862-1959)

Il 20 marzo 1959, all'età di 96 anni, il Prof. GIUSEPPE BASTIANELLI ha chiuso la sua lunga e laboriosa esistenza.

Medico primario degli Ospedali Riuniti, professore di Semeiotica nella Università di Roma e direttore dell'Istituto di Malariologia, da lui creato e dedicato al nome del suo grande maestro ETTORE MARCHIAFAVA, egli era l'ultimo grande rappresentante di quel gruppo di studiosi che verso la fine del secolo scorso si era raccolto intorno al MARCHIAFAVA ed al CELLI contribuendo validamente alla gloria di quella Scuola di Roma cui si deve buona parte delle conoscenze sulla etiologia, la patologia, la patogenesi e la clinica della malaria.

GIUSEPPE BASTIANELLI nacque a Roma il 25 ottobre 1862. Il padre, dottor GIULIO, era primario medico a Roma nell'ospedale di Santo Spirito, nello stesso ospedale in cui poi il Nostro lavorò per vari anni. Uno zio chirurgo in Umbria, luogo di origine della famiglia. Quindi fin da ragazzo egli visse in ambiente medico, cosa che probabilmente indusse sia lui, sia il fratello minore RAFFAELE, ad avviarsi verso la carriera medica, nella quale entrambi dovevano poi raggiungere le più alte vette della fama. Il Nostro è stato uno dei più celebrati medici italiani, il fratello Prof. RAFFAELE, è uno dei più famosi chirurghi che abbia avuto l'Italia.

Durante i suoi studi universitari si dedicò inizialmente con particolare fervore allo studio della chimica, per passare poi a quello della patologia insegnata allora dal MARCHIAFAVA. Scopo dei suoi studi universitari fu quello di crearsi un fondamento solido delle conoscenze di quel tempo, cui aggiungere le nuove che sarebbero venute dopo. Cominciò così fino dai suoi anni giovanili a costruire quell'edificio di dottrina e di cultura che con il volgere degli anni assunse proporzioni sempre maggiori.

Egli è stato uno degli ultimi grandi medici del tipo antico, in grado cioè di abbracciare tutti i campi della patologia e della medicina con competenza tale da sbalordire, fino agli ultimi giorni della sua vita, i più esperti specialisti delle diverse materie mediche; fu un precursore della medicina dei tempi moderni e fin dall'inizio dei suoi studi medici intuì l'importanza sempre maggiore che avrebbe acquistato la fisiologia. Le buone conoscenze di chimica che si era

formate nei primi anni di studio gli riuscirono assai utili per lo studio della fisiologia cui si dedicò con particolare interesse alla scuola del MOLLESCHOTT. Ivi eseguì indagini sui succhi intestinali che stavano per condurlo a conclusioni di grande importanza, ma che non potè proseguire perchè non riuscì ad ottenere i mezzi necessari. Abbandonò così la scuola del MOLLESCHOTT ma non la fisiologia di cui seguì lo sviluppo crescente durante l'intera sua vita

Si dedicò così alla carriera paterna degli ospedali, ove dopo la laurea, conseguita nel 1885, divenne assistente e poi aiuto raggiungendo il posto di primario nel 1891 a soli 29 anni, carriera prodigiosa anche per quei tempi meno difficili degli attuali. La sua attività si svolse per vari anni all'Ospedale di Santo Spirito ove lavorara anche il MARCHIAFAVA, che in quel tempo con ANGELO CELLI si era dedicato particolarmente allo studio della malaria, di cui ALFONSO LAVERAN aveva da poco scoperto l'origine parassitaria. Il Nostro entrò così a far parte di quel gruppo eletto di studiosi cui appartennero anche A. BIGNAMI, A. CELLI, A. GUARNIERI, A. DIONISI ed altri i quali si erano raccolti intorno al MARCHIAFAVA attirati sia dalle sue non comuni qualità di maestro e patologo sia dagli studi sulla malaria che egli conduceva assieme al CELLI.

La scoperta del LAVERAN era stata a lungo oggetto di discussioni e contestazioni, ma il MARCHIAFAVA ed il CELLI ne furono fin dall'inizio strenui assertori e sostennero vivaci polemiche con i detrattori e gli increduli. Il lavoro compiuto dalla Scuola di Roma e da C. GOLGI a Pavia fu prodigioso, tanto più che fu in gran parte eseguito su preparati di sangue a fresco che non consentono se non brevi periodi di osservazione. La tecnica di colorazione dei preparati microscopici era assai poco sviluppata ed anche oggi che tutto è noto sullo sviluppo dei parassiti malarici nel sangue, seguirne le successive fasi nel sangue a fresco richiede esercizio. A. LAVERAN scoprì il parassita, ma la Scuola di Roma e quella di Pavia ne descrissero le varie fasi di evoluzione mettendole in rapporto con le varie manifestazioni morbose.

Raramente i lavori pubblicati dalla Scuola di Roma erano opera di un singolo; il Nostro vi partecipò attivamente lavorando quasi sempre in collaborazione con AMICO BIGNAMI, uno tra i più celebri tra i collaboratori del MARCHIAFAVA. BASTIANELLI era particolarmente interessato ai problemi di neuropatologia ed uno dei primi lavori da loro eseguito sulla malaria tratta appunto di un caso di infezione da *Plasmodium falciparum* con sintomi bulbari associati a disturbi dell'equilibrio, paresi del facciale destro, deviazione della lingua a sinistra, disartria. In successivi lavori descrissero un caso di perniciosa emorragica ed il primo caso di infezione estivo-autunnale con esantema scarlattinoide ed anemia acuta mortale. Eseguirono poi vari studi clinici e parassitologici sulle infezioni da *P. vivax* e da *P. falciparum*, stabilendo anche la durata dell'incubazione nell'infezione sperimentale da *P. falciparum*. Descrissero inoltre la sindrome di corea elettrica manifestatasi in un caso di terzana maligna con anemia gravissima. Le loro osservazioni concorsero validamente a rendere sem-

pre più complete le conoscenze sui parassiti, sulle febbri e le sindromi varie da essi determinate.

Per parecchio tempo dopo la scoperta del parassita, il significato delle semilune nel sangue dei malarici rimase oscuro e fu oggetto di varie interpretazioni. Bastianelli e Bignami fin dall'inizio sostennero che esse fossero forme particolari di evoluzione dei parassiti della terzana maligna non connesse con le manifestazioni morbose dell'infezione. Furono loro a comprenderne in seguito la funzione.

L'argomento della fagocitosi nella malaria fu oggetto di studi molto accurati da parte della Scuola Romana; il Nostro studiò la funzione dei leucociti del sangue e confrontando le proprie ricerche con quelle eseguite sugli organi dal Marchiafava ed altri collaboratori, pervenne alla seguente conclusione: «I leucociti mononucleari circolanti nel sangue si comportano allo stesso modo degli elementi fissi della polpa splenica e midollare con i quali hanno in comune la significazione morfologica».

Da tale affermazione al concetto di sistema reticolo-endoteliale, venuto molti anni dopo, la differenza sta forse solo nel nome. Molte delle cose messe in chiaro dalla Scuola di Roma sono state riscoperte e sono apparse come nuove, mentre erano già note da molto tempo.

Delle varie indagini compiute dal Nostro sulla malaria quelle che gli diedero fama e gloria riguardano il ciclo del parassita nell'anofele. Già nel 1894 Bastianelli e Bignami avevano sostenuto che le semilune andavano considerate come elementi dello stesso tipo di quelle forme di coccidi il cui sviluppo ulteriore si compie nell'ambiente oppure nei tessuti di un altro animale, ma purtroppo non svilupparono subito l'ipotesi da loro affacciata, che li aveva tanto avvicinati alla spiegazione del loro reale significato. Le loro ricerche subirono a questo punto una sosta d'altronde comprensibile tenendo presente che la loro attività non si limitava allo studio della malaria. Come patologi e primari medici di ospedale entrambi erano attratti da vari altri problemi e fu difatti in quel periodo, cioè nel 1896, che il Nostro rese note le sue osservazioni anatomopatologiche sulle sclerosi combinate del midollo spinale nelle anemie perniciose (mielosi funicolare).

Gli studi sulla malaria vennero ripresi in seguito e nel 1898 Bastianelli e Bignami ripresero in esame il problema del significato delle semilune e dei filamenti mobili da esse originati descrivendo la morfologia dei microgameti di *P. falciparum*. Espressero anche l'opinione che lo sviluppo dei gameti avvenisse nelle zanzare. Purtroppo non avevano conoscenze sufficienti sulle zanzare ed ignoravano come se ne eseguisse la dissezione. Si rivolsero così a G. B. Grassi, che svolgeva per conto suo indagini sulle zanzare sospette di trasmettere la malaria, ma aveva inizialmente rivolto la propria attenzione verso *Culex penicillaris* e *C. malariae* che inviava loro in ospedale affinchè li nutrissero con sangue di malarici. I risultati furono naturalmente nulli, ma fortunatamente nell'ottobre 1898 il Grassi, non avendo più potuto catturare le due specie di

Culex, si orientò verso gli anofeli che inviò a BASTIANELLI e BIGNAMI, i quali riuscirono ad ottenere lo sviluppo delle semilune nella zanzara onde il 4 dicembre 1898, con il GRASSI, ne diedero la prima descrizione all'Accademia dei Lincei.

Ma la ricerca della specie di zanzara adatta alla trasmissione della malaria umana aveva richiesto tempo e pochi mesi prima R. Ross aveva descritto lo sviluppo del proteosoma degli uccelli nelle zanzare del genere *Culex*. Ciò diede origine ad una lunga e vivace polemica tra il GRASSI ed il Ross alla quale nè BASTIANELLI nè BIGNAMI vollero in alcun modo partecipare. Il loro scopo era stato quello di ricercare il vettore della malaria umana ed era stato raggiunto; della malaria degli animali non si erano interessati.

Continuando nelle loro ricerche BASTIANELLI e BIGNAMI, dopo avere descritto assieme al GRASSI il ciclo sporogonico di *P. falciparum* nell'anofele, descrissero anche quello di *P. vivax*. Le due descrizioni furono mirabili per la loro completezza, tanto che nulla vi fu aggiunto dopo. Con gli anofeli infatti provocarono l'infezione di un volontario e dettero la prima dimostrazione della trasmissione sperimentale della malaria umana. Si concluse così, gloriosamente, il ciclo di studi sulla malaria della famosa Scuola di Roma. BASTIANELLI contribuì validamente al suo splendore accrescendone i meriti e la fama che aveva conquistata in Italia e nel mondo.

In seguito, per molto tempo il Nostro non si occupò in modo particolare della malaria, dedicandosi alla sua attività ordinaria di clinico e di patologo nella quale eccelse, raggiungendo le più alte vette della perfezione nell'arte medica. La sua preparazione clinica era fondata sulla osservazione anatomica. Ma quando l'indirizzo clinico si orientò verso la fisiologia egli si venne a trovare in posizione di particolare vantaggio, data la sua eccellente preparazione in tale branca della medicina che aveva coltivato fin da giovane ed il cui studio non tralasciò mai durante la vita intera.

Conoscitore profondo dell'anatomia, della fisiologia, dell'anatomia patologica e della neuropatologia, dotato di ingegno ed acume eccezionali, era naturale che la sua figura di clinico emergesse, onde la sua fama si accrebbe tanto che, sebbene egli si ritenesse pago di essere primario negli Ospedali Riuniti di Roma, come lo era il fratello RAFFAELE, gloria della chirurgia italiana, nel 1926 venne sentita l'opportunità di affidargli la cattedra di Semeiotica nella Università di Roma, che egli ricoprì fino al 1935. Sebbene fosse arrivato alla cattedra in età non più giovanile, il Nostro si dedicò con passione all'insegnamento trasmettendo agli allievi il suo immenso patrimonio culturale. Alle dotte lezioni teoriche faceva seguire lunghe ed accurate esercitazioni pratiche e dimostrative su quanto aveva esposto, partecipandovi personalmente. Alla sua scuola si formarono medici valentissimi che oggi continuano la tradizione ed il metodo del loro grande Maestro.

Sebbene la vecchia scuola del MARCHIAFAVA avesse perduto la compattezza di un tempo, i suoi componenti avevano sempre seguito lo sviluppo delle conoscen-

ze sulla malaria ed i risultati del nuovo indirizzo dato alle misure di lotta antimalarica in base alle nuove cognizioni che erano frutto del loro lavoro. Il risultato fu tuttavia modesto e le speranze nutrite della rapida soluzione del problema malarico erano andate sempre più scemando sovrattutto dopo la I^a^ guerra mondiale durante la quale la malaria infierì tra le truppe operanti nonostante la profilassi chininica e varie altre misure preventive adottate. Coloro che avevano assistito a tanto insuccesso sentirono il bisogno di riunirsi per discuterne le cause. Nel 1925 la vecchia Scuola romana indisse in Roma il I° Congresso Internazionale della Malaria che venne presieduto dal MARCHIAFAVA. Il Nostro ne curò l'organizzazione che riuscì oltremodo perfetta; vi parteciparono i più noti e famosi studiosi del problema della malaria. Quel congresso concluse l'attività della famosa Scuola di Roma, ma segnò anche l'inizio di una nuova èra nelle ricerche sulla malaria, che doveva dare tanti notevoli risultati.

Nel 1931 il Nostro fu nominato Direttore della Scuola Superiore di Malariologia, che trasformò nell'attuale Istituto di Malariologia da lui dedicato al nome del suo maestro ETTORE MARCHIAFAVA. Sebbene avesse già raggiunto un'età piuttosto avanzata, egli conservava possibilità fisiche affatto giovanili onde riprese con l'ardore dei vecchi tempi lo studio della malaria e dei suoi problemi. La scoperta degli antimalarici sintetici aveva destato il suo particolare interesse e nel 1933 egli ne analizzò gli effetti paragonandoli a quelli della chinina dei quali aveva larghissima esperienza. In uno studio del 1936 egli confutò, con dati clinici e sperimentali, l'asserzione sostenuta da vari studiosi che la profilassi medicamentosa inibisse la produzione dell'immunità nell'organismo.

Il lavoro svolto dai suoi collaboratori nell'Istituto diede risultati assai soddisfacenti; tra il 1934 ed il 1936 G. RAFFAELE scoprì il ciclo esoeritrocitico dei parassiti della malaria, che colmò l'ultima lacuna rimasta nella loro biologia. Vennero inoltre eseguite varie ricerche di carattere parassitologico, clinico, entomologico ed epidemiologico da lui esposte in una conferenza tenuta nel 1941 all'Accademia medico-fisica di Firenze.

Egli partecipava attivamente ai corsi annuali tenuti nell'Istituto e le sue lezioni erano monumento di dottrina, e di esperienza clinica ed anatomo-patologica.

Durante la sua lunga vita non vi fu alcun argomento della medicina che non sia stato oggetto del suo interesse; dotato di memoria eccezionale, egli ricordava nei più minuti particolari tutto ciò che aveva osservato nella sua carriera di clinico e di patologo o che aveva appreso attraverso la lettura.

Un argomento che lo interessò fino agli ultimi giorni della sua vita fu l'emoglobinuria; da giovane, mentre prestava servizio militare, aveva descritto l'emoglobinuria da marcia da lui osservata in un soldato ed in seguito meditò sempre sulla patogenesi del fenomeno e particolarmente sulla emoglobinuria da malaria. Sull'argomento scrisse moltissimo, ma non dette mai nulla alle stampe, come del resto accadde anche per altri problemi della medicina da lui a lungo studiati.

Non amava pubblicare; un'implacabile autocritica lo tratteneva sempre dal farlo, onde gran parte del suo lavoro è rimasto ignorato.

Amava discutere i problemi che lo interessavano con i suoi collaboratori ai quali leggeva talvolta dei brani dei suoi scritti chiedendo loro il parere con umiltà che sorprendeva in un uomo che non poteva non avere coscienza del suo valore indubbiamente eccezionale. Forse era modesto, certamente non era vanitoso, ed i riconoscimenti che ebbe, sovrattutto la nomina a Senatore del Regno del 1939, gli fecero indubbiamente piacere, ma non li sollecitò e poco gradiva che se ne parlasse. Rifuggiva da ogni forma di pubblicità e non voleva che si richiamasse in alcun modo l'attenzione sul suo nome; non volle neanche che venisse annunciata la sua fine.

La sua vita si concluse così in silenzio, ma la sua grande figura di medico e di studioso rimarrà imperitura a gloria della Scienza Italiana.

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FURTHER OBSERVATION ON THE INTERRUPTION OF MALARIA TRANSMISSION WITH SINGLE DOSE OF PYRIMETHAMINE (DARAPRIM)

M. K. Afridi and A. Rahim (*)

A field trial was undertaken on a group of meso-endemic villages in West Pakistan to test the feasibility of interrupting malaria transmission with a single adult dose of 25 mg. of pyrimethamine during 1957, 1958, 1959. Results indicate a high degree of success for the measure as reflected in the relative figures for infant parasite rates, spleen rates and the number of malaria cases in the test and control villages. The method has considerable potentialities but much ground still remains to be covered before it can be usefully pressed into services for malaria eradication.

In a previous paper (Afridi & Rahim, 1958), we published the results of a field trial to test the effects of a single dose of pyrimethamine (Daraprim) on the transmission of malaria in a group of malarious villages in West Pakistan. Although, the investigations had then lasted but one malaria season of 1957, the resuts obtained were so impressive that it encouraged us to continue the trial over the malaria season of 1958 and 1959. Our study is now complete and for convenience of presentation we have analysed the data of the entire period of test from 1957 to 1959 in this paper.

Epidemiological features of the test villages

The villages selected for trial are located in a sub-montane tract between Haripur and Havelian in the Hazara District of West Pakistan. In this region, malaria usually lasts from the beginning of July to mid-October and is trans-

(*) This enquiry was indertaken as a part of the research programme of North Regional Laboratories, Pakistan Council of Scientific & Industrial Research, Peshawar. We wish to thank Dr. Salim-uz-Zaman Siddiqui, Director, Pakistan Council of Scientific & Industrial Research for his help and suggestions and permission to publish this paper.

mitted by the carrier species, *A. culicifacies*. In the first half of the malaria season *vivax* malaria predominates but by mid-August *falciparum* infections catch up and become dominant by mid-September. Quartan malaria is rare in these parts.

Malaria is notoriously unstable in character displaying wide yearly variations in severity ranging from sharp epidemics to exceedingly mild malaria seasons. The epidemics which usually recur in the region every 7 to 10 years prevail in years of heavy rainfall associated with extensive flooding of the countryside and production of numerous breeding places of *A. culicifacies*. Coincidentally, the atmospheric temperature and humidity remain favourable to malaria transmission for two to three weeks longer in the year of epidemic than in the non epidemic years. Yet another factor in the causation of the epidemic is the low communal immunity resulting from the low incidence of malaria in the inter-epidemic period.

An important feature of the malariometry of these areas is that immediately after an epidemic, there is a steep rise in spleen rates in the entire region affecting children as well as aduts. The rates usually reach 50 per cent or over but the average size of the spleen remains low as would be expected in a non-immune or a partially immune community exposed to a short season of severe malaria. In the inter-epidemic period, trasmission of malaria falls off considerably as the result of which spleen rates undergo a rapid decline in most of the villages. In certain localities, however, particularly those situated near the perennial breeding places of *A. culicifacies* an appreciable amount of malaria transmission persists, and moderately high spleen rates continue during the inter-epidemic period. Such villages collectively constitute the residual « endemic foci » of the region.

This epidemiological picture is too well known to have needed elaboration but we had to refer to it in some detail so as to previde the correct background for our trial and our findings.

Selection of test villages

The selection of suitable villages for a field trial of this nature is always a difficult task but these difficulties are particularly numerous in a region where malaria is unstable. For, in our test area we had to ensure not only a comparable degree of endemicity of malaria in the selected villages but also the fact that in a given year the disease would be equally severe in the different villages. Since these conditions obtain mostly in the residual « endemic foci » our test villages had to be selected from amongst those situated in such localities. We had to look for villages clustered together but care had to be taken that they were not so near each other that one village would directly influence the incidence of malaria in the adjoining one. Finally, since many

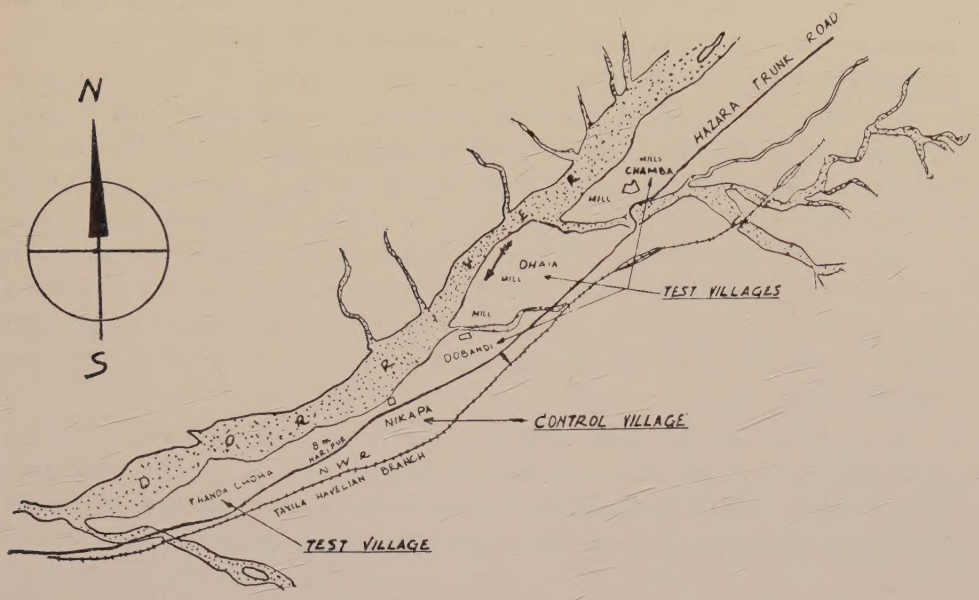

Fig. 1. — Sketch map showing test villages and control village.

of the villages in this region had been subjected to DDT sprayings during 1954 and 1955, we had to exclude them in order to avoid a possible epidemiological complication.

With these criteria in mind we originally selected for our trial a group of four villages namely, Chamba, Dhaia, Dobandi and Nika Pa which are situated on the banks of the river 'Daur' in that order from north-east to south-west as shown in the Sketch Map. In 1958, the trial was extended to yet another village, Thanda Chua south of Nika Pa. These villages formed a compact group within a common epidemiological envoirnment. Spleen rates determined at the start of trial in June 1957 (Table 2) showed that while the figures for different villages varied they were sufficiently high in all to be classified as meso-endemic. The only differential feature was that the northern group of villages comprising Chamba and Dhaia had somewhat lower spleen rates than those of the more malarious southern group.

Administration of drug.

Of the selected villages, Chamba, Dhaia and Dobandi were placed on drug treatment in 1957 whereas Thanda Chua was brought under treatment in 1958. Nika Pa received no treatment and was kept as control throughout the period of the trial.

In 1957 the drug was administered on July Ist but in 1958 and 1959 the treatment was started on 15th of June because of our finding that malaria transmission started earlier than we had previously assumed.

The drug was given in accordance with a procedure which was evolved in 1957 and which was strictly followed in the subsequent years. The dosage scale used was 25 mg. to adults, 12,5 mg. to children from 7 to 12 years of age and 6.25 mg. to children aged 6 years and below. Particulars of each inhabitant of the different villages were entered in registers and the dosage given to each person recorded at the time the drug was administered. This procedure ensured not only a reliable record of pyrimethamine administration, but also provided an up-to-date census of the population under test.

Every effort was made to administer the drug to the maximum number of individuals. In this respect, we were fairly successful, as will be seen from Table I which shows that the percentage of those who received treatment was reasonably high except in the case of Dobandi in the year 1957.

Collection of data

Since the main purpose of the trial was to detect the presence or absence of malaria transmission, it was necessary for us to carry out our investigations on a stable community. Our trial villages fulfilled this condition as most of the inhabitants remained stationary and under continuous observations, and only a small number of families used to move out to the neighbouring town for employment or return to the village after a spell of service. These movements account for variation in the population figures in Table I. From the point of view of spread of malarial infections, however, of greater importance were the ceremonial occasions such as marriages, festivals and deaths when the inhabitants of test villages paid social visits to the adjoining untreated villages and vice-versa.

In our programme of investigations we concentrated on obtaining the data for parasite rates and the incidence of proven malaria cases. While paying due attention to the determination of parasite rates amongst children aged 1 to 9 years we concentrated our efforts more particularly on infants since the presence of parasites in the latter provided a direct proof of malaria transmission. In 1957 we had determined the parasite rates of adolescents of 10 to 16 years but with the exstension of investigations to Thanda Chua and the consequent increase in the load of blood examination we omitted this age group in 1958. In an effort to adjust our work load to the work capacity of the investigation staff we narrowed down the scope of our observations still further in 1959 to (a) infant parasite rates in all the villages (b) parasite

TABLE 1.

Distributiion of Pyrimethamine

Test villages	1957		1958		1959	
	Population	Per cent. received treatment	Population	Per cent. received treatment	Population	Per cent. received treatment
Chamba	601	98.5	613	98.4	607	97.6
Dhaia	274	95.6	334	98.0	305	98.6
Dobandi	186	86.0	225	97.8	247 * 231	98.4 * 93.1
Thanda Chua	—	—	285	98.3	272	96.0
Control village						
Nika Pa	228	—	210	—	216	—

(*) Second round on Ist August 1959 when 16 infants below one year were intentionally excluded.

rates in children 1 to 9 years, in the three villages in the relatively more malarious southern sector namely Dobandi, Thanda Chua and Nika Pa.

Owing to the paucity of trained staff we could not undertake entomological investigations in the early phase of our trial but in 1959 we were able to take in hand the dissection of *A. culicifacies* collected from the control village Nika Pa and the test village Dhaia.

As regards the occurrence of malaria cases, the requisite figures were obtained from a treatment centre and during periodic door-to-door enquiry. The former was established in a centrally placed village which was readily accessible to the other villages, and which served us well not only in attracting malaria cases but also in establishing friendly relations with the local inhabitants. Every patient with fever reporting at this centre was given a single dose of amodiaquine (Camoquine) after a blood slide had been taken. The dosage scale used was 600 mg. of active base to adults; 400 mg. to children from 5 to 15 years; and 200 mg. to children under five years. We selected amodiaquine for clinical treatment because we felt it would not materially disturb the sporontocidal action of pyrimethamine. In the door-to-door enquiry all the villages were visited once a fortnight when fever cases were sought out and given a single dose of « Camoquine » after their blood slide had been taken. These enquiries were started late in the malaria season of 1957 but during 1958 and 1959 they were carried out regularly from July to October.

TABL

Spleen Rates among

	CHAMBA				DHAIA			
	No. Examined	S. R.	A.E.S.	S. I.	No. Examined	S. R.	A.E.S.	S. I.
July 1957	158	15.18	1.5	22,77	79	22.78	1.8	41.00
October	132	3.03	1.0	3.03	81	9.87	1.0	9.87
June 1958	153	0	0	0	90	3.33	1.0	3.33
October	155	0	0	0	91	1.09	1.0	1.09
June 1959	153	2.61	1.0	2.61	78	3.84	1.0	3.84
October	155	1.29	1.0	1.29	83	0	0	0

S. R. = Spleen Rate; A.E.S. = Average Enlarged Spleen;

Analysis of results

The degree of our success in the administration of drug to the test population is reflected in the figures given in Table 1, which show that with the exception of Dobandi 96 to 98 per cent of the inhabitants of all the villages received treatment. In Dobandi the percentage was low (86 per cent) but only in 1957 for in the subsequent two years we succeeded in raising it to 97.8 and 98.4. In 1959, when this village was subjected to a second round of pyrimethamine treatment the drug was taken by no less than 93.1 percent of the inhabitants.

We should perphaps mention that at no time did the villagers object to the taking of the drug although we often had to visit a village twice or thrice on consecutive days to catch the casual absentees. In the case of infants and small children the drug was given from a spoon, a procedure which was greatly facilitated by the sweet taste of «Daraprim».

Spleen rates

Figures for spleen rates in June-July and in October of each year are shown in Table 2 and Graph.

It will be observed that unlike the control village the spleen rates registered a steep fall uniformly in all the test villages which could only have happended either in a state of complete absence or at the most of very low malaria transmission.

ren aged 3 to 9 years.

	DOBANDI			THANDA CHUA				CONTROL VILLAGE NIKA (PA)			
m d	S. R.	A.E.S.	S. I.	No. Examined	S. R.	A.E.S.	S. I.	No. Examined	S. R.	A.E.S.	S. I.
	42.75	1.5	64.13	—	—	—	—	69	40·58	1.4	56.84
	11.53	1.7	19.60	—	—	—	—	65	38.46	1.2	46.15
	3.57	1.0	3.57	93	21.50	1.1	23.65	58	46.55	1.2	55.86
	1.75	2.0	3.50	98	1·02	2.0	2.04	60	53.33	1.3	69.33
	7.81	1.0	7.81	71	7.04	1.4	9.86	44	45.45	1.0	45.45
	1.53	1.0	1.53	87	2.29	1.0	2.29	55	36.36	1.0	36·36

. = Splenometric Index.

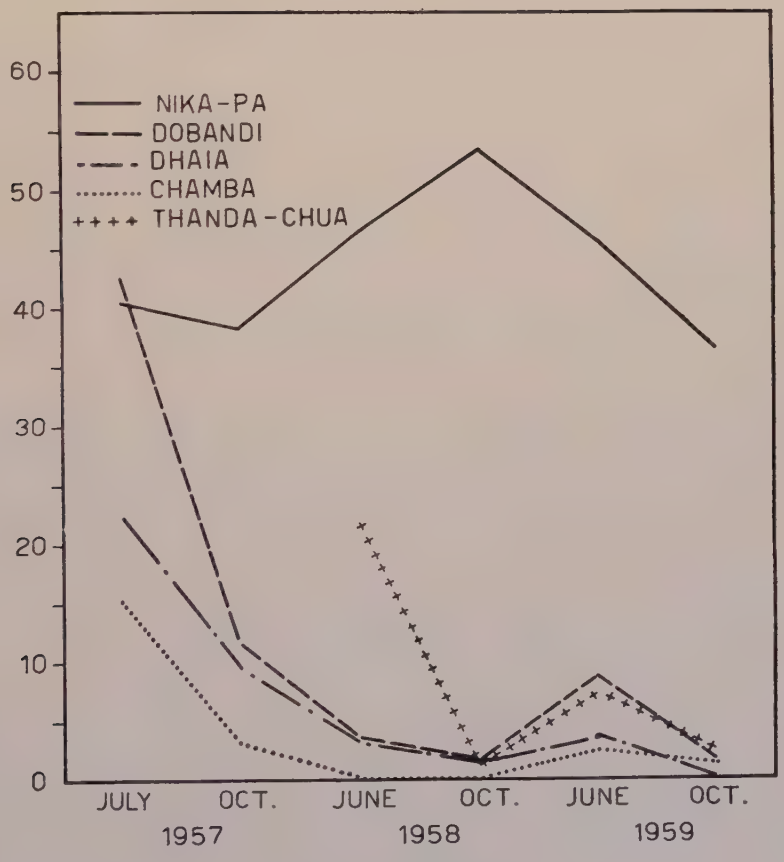

Fig. 2. — Spleen rates.

Infant parasite rates

The number of infants in individual villages was too small to allow of the presentation of our results in percentages vide Table 3. Infant parasite rates in the proper sense of the term could therefore be worked out only for the combined totals of all the test villages.

Table 3.
Infant Parasite Rates.

	1957		1958		1959	
	No. of infants	No. of Positive	No. of infantes	No. of Positive	No. of infants	No. of Positive
Test villages						
Chamba	17	—	12	—	25	—
Dhaia	8	—	14	—	5	1
Dobandi	7	1	7	—	6	—
Thanda Chua	—	—	3	—	8	—
Total test villages	32	1	36	—	44	1
Infant parasite rates . . .		3.1		0		2.3
Control village						
Nika Pa	4	3	8	6	5	3

From a study of Table 3 it will be seen that in the test villages only two infants were found positive out of 112 infants examined in the course of three years as against 12 infants out of 17 in the control village. This finding provides a convincing proof of not only the protective value of pyrimethamine but also of the intense degree of transmission that must have prevailed in the experimental area.

Of the positive infants in tests villages one was found in Dobandi on 7th September, 1957, while the other was encountered in Dhaia on 17th August, 1959 i.e. on the 69th and 63rd day after the distribution of pyrimethamine respectively. Allowing for the incubation periods in man and mosquito, the potent gametocytes that gave rise to these infections must have appeared on or about the 42nd and 36th day after the drug was administered.

In the control village Nika Pa, infections amongst infants were found throughout July, August, September and October. A noteworthy feature of these infections was that 5 out of 12 positive infants harboured *P. falciparum*, in contrast to the test villages where both the infections were *P. vivax*. Another feature worthy of note was that infection in infants in the control village was found as early as 2nd July in 1957 and 1958 indicating that malaria transmission began atleast three weeks earlier in our experimental area than in the neighbouring plains.

Parasite rates in children 1 to 9 years and incidence of malaria cases

Parasite rates in children aged 1 to 9 years are given in Table 4 while total malaria cases recorded in the treatment centre and door-to-door enquiry are presented in Table 5.

We have intentionally taken up together the consideration of these two sets of figures as we feel that it might be misleading to draw conclusions from one Table separately particularly as the provision of prompt and effective

Table 4.

Parasite Rates in Age Group 1 to 9 years.

Year & months	TEST VILLAGES								Control village	
	Chamba		Dhaia		Dobandi		Thanda Chua		Nika Pa	
	No. examined	Parasite Rate	No. examined	Parasite Rate	No. examined	Parasite Rate	No. examined	Parasite Rate	N. examined	Parasite Rate
1957										
July	195	5.13	94	4.26	44	6.82	—	—	65	10.77
August	138	3.62	60	6.67	49	6.12	—	—	59	16.95
Sept.	161	2.48	80	5.00	58	12.07	—		47	23.40
October	160	0.63	97	0.00	63	3.17	—	—	7	10.29
1958										
June	192	0.00	105	0.00	64	0.00	111	0.90	66	4.54
July	154	0.00	85	0.00	58	1.70	98	0.00	62	6.45
August	173	0.00	89	1.12	60	3.33	92	2.17	57	7.01
Sept.	157	1,91	94	1.06	58	3.44	102	0,98	62	3.22
October	163	1.22	96	2.08	61	1.63	104	0.96	55	0.00
1959										
June	172	1.16	96	0.00	76	2.63	96	1.04	68	5.88
July	—	—	—	—	56	3.57	84	0.00	50	4.00
August	—	—	—	—	64	1.56	81	0.00	60	6.67
Sept.	—	—	—	—	60	1.67	77	1.30	64	6.25
October	169	4.73	100	1.0	58	1 72	85	2.35	61	11.48

treatment for malarial attacks had a markedly disturbing influence on the parasite rates. Thus in the control village the parasite rates were higher in 1957 than in 1958 and yet judging from the occurrence of malaria cases, 1958 had a definitely severer malaria season than 1957. Indeed, because of the steep rise in malaria during July and August, we seriously apprehended the occurrence of an epidemic in 1958 which was, however, averted by a timely rainfall in August that flushed away the breeding places of *A. culicifacies* in the river « Daur ». The curve of parasite rates in 1959 similarly fails to portray the special features of that year namely the delayed start of malaria

TABLE 5.

Total Malaria Cases recorded in Treatment Centre and Door-to-Door enquiry.

Months & year	Test Villages				Control Village
	Chamba	Dhaia	Dobandi	Thanda Chua	Nika Pa
1957					
July	0	0	0	—	1
August	1	1	1	—	8
Sepetember	3	4	12	—	24
October	1	0	2	—	10
Total cases	5	5	15	—	43
Population	601	274	186	—	228
Ratio per 1000	8.32	18.25	80.65	—	188.60
1958					
July	0	0	1	0	7
August	1	1	2	2	23
September	2	2	5	1	34
Ocotober	5	3	9	2	15
Total cases	8	6	17	5	79
Population	613	334	225	285	272
Ratio per 1000	13.05	17.96	75.56	17.54	290.44
1959					
Iuly	0	2	1	1	10
August	2	2	1	1	2
September	5	7	3	2	18
October	9	2	3	3	11
Total cases	16	13	8	7	41
Population	607	305	247	272	216
Ratio per 1000	26.36	42.62	32.39	25·74	189.81

season and a lower level of malaria incidence rate than in the preceding two years.

In view of these findings in the control village, the interpretation of parasite rates of the test villages has to be undertaken with the utmost caution. Nevertheless, the fact that in test villages these rates were about 1/2 to 1/3 of the rates in the control villages is a finding of some significance.

A clearer indication of the beneficial effects of phyrimethamine is furnished by a comparison of the number of malaria cases in the control and test villages. For convenience of presentation we have recast these figures in Table 6 and which show that malaria cases in the test villages were approximately 1/8th, 1/12th and 1/6th of the number in the control village in 1957, 1958 and 1959, respectively.

TABLE 6.

Total Malaria Cases.

Year	Total Cases in Test Villages			Cases in contro village		
	Total Population	Nomber of cases	Ratio per 1000 population	Population	Number of cases	Ratio per 1000 population
1957	1061	25	23·56	228	43	188.60
1958	1457	36	24.71	272	79	290.44
1959	1431	44	30.76	216	41	189.81

DISSECTION OF *A. culicifacies*

As already stated we were able to undertake the dissection of *A. culicifacies* only in 1959, the results of which are presented in Table 7.

TABLE 7.

Dissection of A. culicifacies.

	Control village Nika Pa				Test village Dhaia			
	Number dissected	Number positive			Number dissected	Number positive		
		Oocysts	Sporozoites	Percent.		Oocysts	Sporozoites	Percent.
August	134	0	0	0	183	0	0	0
September	230	3	0	1.47	229	0	0	0
Total . .	364	3	0	0.82	412	0	0	0

Considering that the infection rates in *A. culicifacies* are apt to be particularly low during the inter-epidemic period, it is not surprising that our investigation should have yielded so few positive results. However, the presence of three gut infections in the control village provides valuable evidence that confirms our findings in the other areas of this enquiry.

DISCUSSION

We attach special importance to the fact that this investigation was addressed expressly to the question whether malaria control could be effected exclusively through the agency of a sporontocidal drug. This concept is admittedly not new as plasmoquine was tried for the same purpose over

twenty years ago. In recent times too, the valuation of anti-malarials is reckoned not only in terms of their schizontocidal action but also of their action on gametocytes. In practice, however, drugs occupy a secondary position in anti-malaria schemes, their use being restricted mainly to the clinical or radical cure of malarial attacks or in special circumstances to suppressive treatment.

Sporontocidal action of drugs, though widely acclaimed, is scarcely ever exploited and is utilised merely as an adjuvant to the traditional anti-malarial measures. This attitude, which is traceable primarily to the difficulties associated with the distribution of drugs in rural areas, was fully justified in the case of suppressive treatment when the drug had to he administered daily or once a week or at the most once a fortnight. The use of an anti-malarial as sporontocide is, however, a radically different proposition inasmucn as in most areas treatment for this purpose need only be given once or twice in the malaria season. Elaborate arrangements will still be necessary to provide proper supervision over drug administration but these will be no more exacting than the organization necessary for a properly conducted DDT spraying operation.

These considerations prompted us to test in the field the efficacy of pyrimethamine as a sporontocide. In planning our trial, however, we had to provide such optimum conditions as would ensure for pyrimethamine the best chances to produce results. In the first place, the drug had to be administered before the start of the malaria season. In our trial the start was definitely late in 1957 while in 1958 and 1959, the drug would have been more effective had we started its distribution two weeks earlier than we actually did. Secondly, the drug had to be administered to the maximum number of inhabitants and precautions had to be taken to ensure that they actually swallowed it. Owing to the limited nature of our commitment, we did not encounter much difficulty on this score but as already stated, elaborate arrangements will have to be made in a large scale anti-malaria scheme. Thirdly, the dosage of the drug had to be adequate. We selected 25 mg. dose because of a communication from Dr. G. R. Coatney (1957) to the effect that « on a calculated basis following a single 25 mg. dose of pyrimethamine, there would still be a suppressive amount (i.e. 0.8 mg. of the drug) in the body after 52 days ». On actual trial, however, a definite break through occurred with this dosage on two occasions on 69th and 63rd day after treatment indicating that 25 mg of pyrimethamine had ceased to be fully effective against gametocytes after about 42 and 36 days respectively. These results may be compared with those of Roberts (1956) in East Africa who observed a significant rise in parasite rates 12 weeks after mass treatment with a single adult dose of 50 mg. of pyrimethamine. The viable gametocytes

that gave rise to the infections in the African experience probably appeared about 8 weeks after treatment i.e. 2 to 3 weeks later than in our trial.

Despite this limitation, however, the malariometric data indicate a very high measure of success for our trial. This is evident from the precipitous fall in the spleen rates, the low infection rates in infants and the small number of proven case of malaria in the test villages as compared with the state of affairs in the control village. The notable achievement in these respects should not however lead us to overlook the fact that in our test area with a short malaria season of four months, a single dose of 25 mg. of pyrimethamine did not abolish malaria completely even after treatment over three consecutive years. In 1959 we administered a second dose of pyrimethamine to the inhabitants of Dobandi six weeks after the first. This brought down the number of malaria cases in the village to less than half of the numbers in the preceding two years, but a few cases still occurred there due either to insufficiency of the dosage or to infections acquired during social visits of the test villagers to the untreated villages.

Conclusions

While our trial may claim credit for pointing up the feasibility of utilising a sporontocidal drug for anti-malaria purposes in the field, we realise that much ground still remains to be covered before this method can be pressed into service for malaria eradication. The most urgent requirement in this regard is to work out a suitable scale of dosage based on the degree of efficacy of the drug on one hand and the length of the malaria season on the other. The aim should be to fix a dosage which will necessitate one or at the most two rounds of drug administration in a malaria season. The next important need is to initiate a search for new sporontocidal drugs with longer periods of effectiveness than the existing ones. Improvement in these directions will not only increase the practical value of this measure in the field but will go a long way towards solving the problem of malaria eradication in areas where anophelines have become resistant to more than one group of insecticides.

ULTERIORI OSSERVAZIONI SULLA INTERRUZIONE DELLA TRASMISSIONE DELLA MALARIA MEDIANTE UN'UNICA DOSE DI PIRIMETAMINA (DARAPRIM).

1) Un esperimento per saggiare in pratica la possibilità di interrompere la trasmissione della malaria mediante l'azione di un preparato sporontocida è stato effettuato per tre anni consecutivi in una piccola comunità vivente in un'area mesoendemica. Le dosi impiegate sono state: 25 mg. di pirimetamina (Daraprim) agli adulti, 12,5 mg. ai bambini da 7 a 12 anni, e 6,25 mg. ai bambini di 6 anni o sotto questa età.

2) I dati malariometrici sono indicativi di un notevole successo dell'esperimento, come si rileva dalla ripida caduta nel tasso parassitario, dal basso grado di parassitismo infantile e dalla bassa incidenza dei casi di malaria. Con il dosaggio impiegato non si è tuttavia verificata la cessazione della trasmissione per l'intera stagione. Insuccessi sono stati osservati in due occasioni ponendo in evidenza il fatto che l'efficacia di 25 mg. di pirimetamina perdurava un minimo di 42 e di 36 giorni.

3) Si conclude che, mentre è possibile raggiungere una considerevole riduzione della malaria con una dose di 25 mg., per l'eradicazione sarà richiesta una dose molto più elevata che necessariamente sarà stabilita da ulteriori indagini.

4) I preparati sporontocidi meritano di essere appropriatamente utilizzati e sviluppati poichè possono risolvere il grave problema della eradicazione della malaria nelle aree in cui gli anofelini sono divenuti resistenti a più di un gruppo di insetticidi. In accordo a ciò, la cosa più urgente è di cercare nuovi preparati sporontocidi con un periodo di efficacia più lungo di quello della pirimetamina.

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ACTION SPORONTOCIDE ET CLINOPROPHYLACTIQUE DE LA PYRIMÉTHAMINE

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L'étude sur 72 impaludés sur l'action sporontocide séléctive de la pyriméthamine seule ou en association avec les 4 et 8 amino-quinoléïnes dans la cure radicale du paludisme a etabli: 1, qu'une dose unique de 25-50 mgr. de pyriméthamine n'excerce pas d'effet parasiticide contre les sporozoïtes ni contre le formes preérythrocytaires de *P. vivax, P. malariae* et *P. falciparum;* 2, une dose unique de pyriméthamine, seule ou en association avec la chloroquine, exerce une action inhibitrice sur le cicle sporogonique des mêmes éspèces en 100% des cas.

Le plan des essais de Chimiothérapie — coordonnés par la Direction de l'éradication de l'OMS (1956-1957) « sur au moins 20 sujets présentant des infections à *P. vivax* et, si possible sur un petit nombre de sujets présentant des infections à *P. falciparum* et sur un petit nombre de sujets présentant des infections à *P. malariae* » a été étendu à un rapport avec le spécifique local des méthodes d'impaludation thérapeutique du Centre Berceni. La variante locale du schéma — imposée autant par les souches de parasites utilisées et l'état général de résistance de patients que par les conditions même d'hospitalisation et de contrôle — comporte:

A. *P. vivax* (1) l'étude sur l'*action sporontocide* à dose unique de pyriméthamine seule ou associée à la chloroquine sur 11 impaludés à *P. vivax* — souches K. M. et V; (2) essais de Clinoprophylaxie d'une dose unique de pyriméthamine sur 8 impaludés par infections répétées de sprorozoïtes.

B. *P. falciparum* (1) effets sporontocides et schizontocides d'une dose unique de pyriméthamine seule ou associée à la cloroquine sur 15 impaludés à sporozoïtes ou au sang virulent de *P. falciparum.* (2) essais sur l'effet clinoprophylactique d'une dose unique de 50 mgr. de pyriméthamine administrée à 3 impaludés.

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C. Sur le nombre total des 377 malades 26 ont reçu à la sortie de l'hôpital soit la cure radicale schizonto-gamétocytocide « de sûreté » soit une association de pyriméthamine + 4 et 8 aminoquinoleine pour une durée de 3 à 5 jours.

D. Des essais de cure radicale — soit à l'aide de schizonto-gamétocytocides, soit de fortes doses de pyriméthamine + chloroquine — ont été également effectués sur 35 impaludés:

: 4 à *P. vivax;* : 24 à *P. falciparum;* : 7 à *P. malariae.*

Technique. L'impaludation thérapeutique a été effectuée respectivement à l'aide de : 3 souches, de *P. vivax* — K; Mr; V; préalablement étudiées au point de vue: — virulence, immunogénèse; capacité gamétogène.

: 1 souche de *P. falciparum* R. Fil;

: *P. malariae* W. A.

Contrôle journalier : hématoparasitaire, fièvre, état général et fonctionnel. Après un certain nombre d'accès — réclamés par la pyrétothérapie — on administrait aux « *porteurs de gamétocytes* » — infectants pour *A. atroparvus* (colonie d'insectarium) — *la dose unique de médicament expérimenté.*

L'infectivité du sang pour le vecteur était déterminéee:

(1) avant le traitement; (2) à différents intervalles après le traitement jusqu'à la disparition du parasite du sang.

Les lots d'anophèles gorgés étaient maintenus à 25-27° C et 60% - 30% humidité. La dissection des moustiques à partir du 5-e jour était continuée jusqu'à l'épuisement du lot.

Le contrôle des rechûtes des malades effectué jusqu'au départ de l'hôpital marquait des périodes variables de 20 à 370 jours.

Dans les essais de Clinoprophylaxie: (a) on administrait une dose unique de 25 à 50 mgr de pyriméthamine aux malades préalablement examinés fonctionnellement, (b) l'administration du médicament était suivie à des intervalles d'*inoculation répétés de suspensions de sporozoïtes* ou *piqûres d'anophèles infectés* avec les souches de plasmodium cités ci-dessus.

Résultats

I. Impaludation à *P. vivax.*

A) *Effet sporontocide et schizontocide d'une dose unique de pyriméthamine, employée seule ou en association avec la chloroquine.*

Parmi les 11 malades (du groupe a), neuf neurosyphilitiques ont été impaludés par inoculation de sporozoïtes de la souche « K »; un malade avec la souche locale « Mr » et le onzième avec la souche locale « V » de *P. vivax.* Après l'apparition des gamétocytes, on a administré à deux des sujets (BV/1; D. 1/2) une « dose unique de pyriméthamine seule (25 à 50 mgr) et aux

neuf autres (B.I.3; C.G.4; V.S.5; O.R.6; U.E.61; R.A.84; D.F.85; V.N.94 et D.G.99), une « dose unique » contenant 25 mgr. de pyriméthamine et 300 à 600 mgr. chloroquine base.

Les effets du traitement sur la persistance de la fièvre, sur les trophozoïtes et sur les gamétocytes ont été notés, et l'on a déterminé l'infectivité des gamétocytes pour les moustiques, avant et après le traitement, en laissant des *A. atroparvus* se gorger sur les malades, porteurs de gamétocytes.

Les résultats acquis montrent que chez les moustiques qui s'étaient nourris sur les malades — à des intervalles d'une à 24 heures avant l'administration du médicament, — 20 à 100% contractaient l'infection, tandis que, *parmi ceux nourris sur les malades, 4 et 24 heures après le traitement, aucun n'a été infecté; absence d'oocystes à la dissection.*

Dans 5 de ces cas, les repas de sang répétés 3 et 7 jours plus tard *n'ont pas été suivie d'infection,* mais les densités de gamétocytes étaient alors « très faibles »; 4 à 16 moustiques seulement ont été disséqués.

Au cours d'une observation de durée variable après la maladie, 3 malades V.S. (5); O.R. (6) et V.E. (6') *ont présenté des rechûtes* à des intervalles de 220/91 et 211/43; 272/85 jours respectivement après le traitement à doses uniques — I et II. —

En résumé, les résultats des essais concernant l'activité inhibitrice de la pyriméthamine — seule ou associée à la chloroquine — sur le « cycle sporogonique » du *P. vivax* confirment les données des autres malariologistes. Le sang de 11 porteurs de gamétocytes, qui était infectant pour *A. atroparvus* de une à 24 heures avait le traitement à dose unique, *ne l'est plus après le traitement.* Dans les conditions de « ces essais » *la dévitalisation évidente des gamétocytes* persiste jusqu'au 7e jour après l'administration du traitement à dose unique, coincidant, il est vrai également avec une très faible densité de la *gamétocytémie,* d'une part et absence d'oocystes dans les dissections des anophèles gorgés.

L'effet *schizontocide* relativement lent — mais néanmoins constant — de la pyriméthamine « seule » est visiblement accéléré par association de la la chloroquine. Les effets parasiticides des deux substances sur les formes *erythrocytaires* du parasite sont nettement vérifiée.

L'apparition des rechûtes « tardives » en 3 sur les 11 cas (jusqu'à cette date) après respectivement: (1) 220, 211 et 272 jours, suivant la première dose unique de pyriméthamine — seule ou associée à la cloroquine; (2) 91, 42 et 85 jours après une seconde « dose unique » met en évidence nette « l'absence d'action de la pyriméthamine sur le *cycle exoérythrocytaire* tissulaire tardif du parasite ».

Une seconde rechûte tardive du malade O.R.$_6$ 46 jours après « une 3-e dose unique » confirme entièrement cette hypothèse.

B) *Durée de l'effet clinoprophylactique d'une dose unique de pyriméthamine.*

On a administrè à 6 malades: 1/D.V.$_{7}$; 2/I.D.$_{8}$; 3/J.N.$_{9}$; 4/L.N.$_{98}$; 5/S.N.$_{90}$ 6/A.G.$_{91}$ une dose unique de 25 ou 50 mgr. pyriméthamine; le même jour et plusieurs jours plus tard on les a infecté avec *P. vivax* (souche « K ») en inoculant des sporozoïtes à trois malades: 1/D.V.$_{7}$; 2/I.D.$_{8}$; 3/J.N.$_{9}$. Les inoculations — effectuées soit par injection intraveineuse d'une suspension de sporozoïtes, soit par piqûre de 1 à 4 *A. atroparvus* — ont été renouvelées à des intervalles d'environ une à deux semaines jusqu'à l'apparition de parasites dans le sang.

Le malade — 4/L.N.$_{98}$ — a reçu, 5 jours après l'administration de la pyriméthamine, des inoculations à sporozoïtes, durant 3 jours de suite. Au bout 89 jours, traitement de sûreté avec chloroquine + plasmocide 3 jours. Rentré à l'hôpital le 26.XI, presente une rechûte clinique et parasitaire, 255 jours après l'infection.

Les malades — 5/S.N.$_{90}$; 6/A.G.$_{91}$ — impaludés respectivement 24 h. et 7 jours après administration de pyriméthamine, à des intervalles variant de trois semaines à deux mois — ne présentaient pas de parasitémie après 59 et 47 jours d'observation respectivement.

Les résultats de cette expérience, suggèrent *une durée variable de protection clino-prophylactique du médicament, de 47 à 89 jours.*

Deux autres malades P. V. et D. M. qui ont reçu 24 heures, après la dose unique de 25 mgr pyriméthamine une inoculation de sang virulent, n'ont pas présenté de parasitémie — AoPo — 15 à 30 jours d'observation après l'inoculation.

En résumé: Les résultats des essais de « prophylaxie causale d'une dose unique » de 25 mgr à 50 mgr de pyriméthamine administrée à 6 paralytiques avant l'impaludation à sporozoïtes unique ou répétée à des intervalles de 1 à deux semaines, démontrent:

(a) La pyriméthamine n'a pas d'action sur le *sporozoïte* et sur les formes pré-érythrocytaires du parasite;

(b) Son action sur les formes *érythrocytaires* du parasite est démontrée par une durèe variable de clino-prophylaxie contrôlée — AoPo — respectivement de: — 83 (D. V.) —; 65 (I. D.) —; 54 (J. N.). — Les 3 autres malades qui avaient reçu un traitement schizonto-gamétocytocide de « départ » ont présenté la maladie clinique et parasitaire respectivement après 255, 278 et 255 jours après la 1-ère impaludation.

(c) Afin d'utiliser les remarquables qualités — suppressive et anti-sporogonique — de cette substance, nous proposons en clino-prophylaxie comme marge de sûreté pendant la saison épidémique l'addition de 25 mgr pyriméthamine toutes les 3 ou 4 semaines à la dose hebdomadaire de 300 mgr chloroquine.

II. Infection à *P. falciparum* — souche «R» Fil.

Cette souche a été isolée à partir d'une rechûte survenue dans un microfoyer résiduel d'infection. Au début des expériences, elle avait subi 13 passages par inoculation de sang.

A) *Effet sporontocide et schizontocide d'une dose unique de pyriméthamine employée seule ou en association avec la chloroquine.*

Des impaludations «en but de traitement» au moyen de cette souche ont été provoqués:

(a) chez 15 malades: 10: — L.I.13; C.T.62; D.S.67; I.N.80; Z.N.83; C.P.86; A.G.87; M.P.89; O.R.89; O.R.92 et C.I.93 — par injection de sang virulent; (b) les autres 5: (C.E.65; D.T.70; B.A.81; B.C.82; V.S.88), étaient impaludés par inoculation de sporozoïtes.

Le traitement par «dose unique» a été administré entre le 5-e et 24-e jour ayant suivi l'apparition des gamétocytes: cinq malades — 13; 65; 70; 86 et 92 — ont reçu une dose unique de 25 à 50 mgr «pyriméthamine seule»; 10 malades: — 62; 67; 80; 81; 82; 83; 87; 88; 89 et 93 — une dose de 25 mgr «pyriméthamine + 300 à 600 mgr chloroquine».

Des lots d'*A. atroparvus* qui s'étaient nourris sur les malades, de 1 à 24 heures *avant le traitement,* se sont infectés dans des pourcentages compris entre 11 et 72%; dans 2 cas seulement: — no. 1 et 14 —, le pourcentage d'insectes infestés a atteint 100%.

Aucune des moustiques s'étant gorgés sur les malades 2 à 24 heures après l'administration du mèdicament n'a été infecté. Les repas de sang ont été renouvelés à divers intervalles, après l'administration du médicament, respectivement: une semaine dans 8 cas; 2 semaines en 4 cas; 3 semaines en 3 cas. *Aucun moustique n'a été trouvé infecté.*

B) *Durée de l'effet clinoprophylactique d'une dose unique de pyriméthamine dans l'infection à P. falciparum.*

On a essayé l'action clinoprophylactique de la «dose unique» de 50 mgr de pyriméthamine chez trois malades: 95 R. M.; 96 C. G.; 97 U. F.

Deux malades ont reçu une seule infection à sporozoïtes, 24 heures et 7 jours respectivement après l'administration du médicament, le troisième recevant, 7 jours après la pyriméthamine, trois inoculations successives de sporozoïtes, à des intervalles de 7,8 et 11 jours. Les trois sujets n'ont pas présenté la maladie — en cours d'observation — de respectivement: 92, 186 et 141 jours après l'administration de la dose unigue de pyriméthamine.

Essais de cure radicale — traitement combiné: schizonto-gamétocytocide et pyriméthamine

Des conditions particulièrement précaires d'état géneral et de moindre résistance de 35 *paralytiques,* qui réclamaient — au cours de la pyrétothérapie

— l'interruption rapide de l'accès et, par la suite, la cure radicale à l'aide d'un *traitement schizonto-gamétocytocide,* permettent une évaluation des méthodes utilisées en associant: — l'effet schizontocide lent de la pyriméthamine sur les *formes erytrocytaires* avec l'action schizonto-gamétocide rapide des: 4 et 8 aminoquinoléines combinés.

A. *Impaludation à sang virulent de* P. vivax *souche « K ».*

Malades — R. P.$_{14}$ et D. T.$_{15}$ — ayant pris respectivement au 16e et 19e jour de l'infection 25 *mgr. pyriméthamine* 3 *jours de suite* — étaient encore porteurs de parasites (AoP+) le 5e et 3e jour, respectivement après la première dose; les parasites ont été éliminés (AoPo) dans les premières 24 heures de traitement schizonto-gamétocide chloroquine + plasmoquine × 3 jours. On ne signale pas de rechûtes après 686 jours d'observation du malade D.T.$_{15}$, encore à l'hôpital le 15-II-1959.

Chez les malades — T. S.$_{18}$ et I. G.$_{19}$ — ayant reçu d'emblée — respectivement au 22e et 37e jour de l'infection — *un traitement combiné de* 3 *jours « pyriméthamine + chloroquine-plasmoquine »* est suivi d'effets parasiticides — AoPo — dans les premières 78 heures après le début du traitement.

B. *Impaludation à sang virulent de* P. falciparum *souche « R-Fil ».*

Des observations effectuées sur 24 *impaludés,* dont l'état général réclamait l'interruption de l'impaludation, mettent en évidence les particularités thérapeutiques des différents médicaments et méthodes employées:

a) Malade — A.I.$_5$, traité au 33e jour de l'infection avec *« une dose unique de* 50 *mgr. pyriméthamine »* presente une rechûte — A+ P+ — 16 jours après l'administration du médicament. Une seconde dose de 50 mgr. pyriméthamine administré au malade — porteur de gamétocytes — (24 jours après la première dose) ne fait pas disparaître les gamétocytes; le malade n'en est débarassé — AoPo — *que* 12 *jours après un traitement schizonto-gamétocide* de « precaution » à 300 *mgr paludrine pendant* 7 *jours associée* 0.02 *gr. plasmoquine* × 5 *administrée en* 5 *jours de suite.*

b) Malade semi-immun — I. M.$_7$ —, traité le 29e jour de l'infection, avec *une « dose unique de* 50 *mgr. pyriméthamine »*:

— les trophozoïtes sont disparu rapidement, les gamétocytes persistent dans le sang 15 jours même après une *seconde dose de* 50 *mgr. pyriméthamine* (administrée 22 jours après la première). Le malade est en observation.

c) Chez le malade — B. I.$_6$, — traité le 19e jour de l'infection — avec *« une dose unique de* 50 *mgr. pyriméthamine »* les trophozoïtes ont disparu 3 jours après; les gamétocytes ne disparaissent qu'après *un traitement de* 3 *jour à la « chloroquine* 1200 *mgr. associée à la plasmoquine* 0,06 *gr »* (au total).

d) L'association « pyriméthamine + chloroquine » accélère de manière évidente l'action schizontocide et diminue la période de la gamétocytémie

chez 9 malades — N.N.$_8$; Z.S. $_9$; I.A.$_{10}$; I.I.$_{11}$; E.I.$_{12}$; P.P.$_{13}$; P.I.$_{50}$; I.G.$_{51}$; B.B.$_{54}$.

Le malade N.N.$_3$, traité le 22e jour de l'infection avec « 75 *mgr. pyriméthamine* + 1500 *mgr. chloroquine* » en 3 jours; les trophozoïtes disparaissent en 2 jours et les gamétocytes persistent encore 28 jours après.

Les derniers *huit malades* traités du 14e au 26e jour de l'infection à l'aide d'une « *dose unique de* 25 *mgr. pyriméthamine associés à* 600 *mgr. chloroquine* »; effet anti-trophozoïte en 2 à 3 jours; les gamétocytes persistent de 16 à 28 jours dans le sang des malades: — Z.S.$_9$; I.A.$_{10}$; I.I.$_{11}$; E.I.$_{12}$; P.P.$_{13}$.

Les trois autres, en quittant l'hôpital, ont reçu différents traitements schizonto-gamétocides « de précaution » après la dose unique associés:

1) Le malade P.I.$_{50}$, ayant reçu 12 jours après, une dose de plasmoquine 0,02 gr. × 5 jours; les gamétocytes ont disparu 2 jours après;

2) le malade — I.G.$_{51}$ —, ayant reçu les jours après, 1200 mgr. chloroquine + 0,06 gr. plasmoquine (en 3 jours); la disparition des gamétocytes 5 jours après;

3) le malade — B.M.$_{54}$ —, ayant reçu 9 jours après la dose unique « pyr. + chlr » — du paludrine 300 mgr. × 7 jour — associée à 0,01 gr. plasmoquine × 5 jours; les gamétocytes ont disparu 4 jours après;

4) la sang du malade P.P.$_{13}$, traité à l'aide de la « dose unique de pyriméthaine associée à la chloroquine » est *non-infectant par transfusion* à un nouveau malade, 14 jours après la disparition des gamétocytes. Ce fait confirme la valeur de la *méthode associée,* utilisée dans le cure radicale sur le terrain.

e) Chez 3 malades — G.N.$_{57}$; S.G.$_{55}$; P.V.$_{56}$ — ayant reçu respectivement le 11e, 18e et 31e jour de l'infection — une quantité totale de chloroquine 1500 mgr. associée à la plasmoquine 0,06 gr. distribuée en 3 jours; les throphozoïtes ont disparu en 2 à 3 jours, les gamétocytes 3 à 6 jours après.

f) Trois malades — S.G.$_{64}$; P.C.$_{63}$ et V.R.$_{56}$ —, traités respectivement au 13e, 23e et 13e jour de l'infection à l'aide de moitié dose de « *chloroquine* 750 *mgr. associés à la plasmoquine* 0,06 *gr.* » en 3 jours; les *trophozoïtes ont disparu* 1 *à* 2 *jours; les gamétocytes* 4 *à* 5 *jours* après le début du traitement

g) Quatre malades: R.M.$_{60}$; A.M.$_{59}$; I.C.$_{58}$; et I.O.$_{61}$ —, traités respectivement le 11e, 12e, 13e et 16e jour de l'infection à l'aide de « *paludrine* 300 *mgr. pendant* 7 *jours associée à la plasmoquine* 0,02 *gr. pendant* 5 *jours* », les trophozoïtes disparaissent en 3 à 4 jours; les gamétocytes en 5 à 10 jours après le début du traitement.

h) Deux malades: A.E.$_{52}$ et N.H.$_{53}$ —, traités respectivement le 21e et 31e jour de l'infection — par *la quinine* 1 *gr. par jour, pendant* 7 *jours,* suivie de *plasmoquine* 0,02 *gr. pendant* 5 *jours,* les trophozoïtes ont disparu après 2 et 3 jours, les gamétocytes 13 jours après la quinine, chez le malade A.E.$_{52}$ et 5 jours après la plasmoquine, chez le malade N.H.$_{53}$.

C) *Impaludation à sang virulent de* P. malariae *souche AW.*

Cette souche d'origine africane, actuellement au 368 passage, par sang virulent, est particulièrement préférée en impaludation thérapéutique pour son agaméthogenèse et ses qualités pyréthogènes constantes mieux supportées par les malades.

On a utilisé comme traitement des fortes doses répétées de pyriméthamine seule ou associée à la chloroquine, dans le traitement de 7 malades (D.M.[16]; D.G.[17]; S.M.[30]; E.G.[21]; S.D.[22]; H.H.[23] et S.J.[24]).

1) Malade — D.M.[16] —, traité le 32[e] jour de l'infection avec doses répétées de 75 *mgr. pyriméthamine pendant* 4 *jours,* la parasitémie diminuée, n'a disparu qu'après une dose de 300 mgr. chloroquine associée à 0,02 gr. plasmoquine.

2) Malade — D.G.[17] — traité le 30[e] jour de l'infection avec 100 *mgr. pyriméthamine,* dose répétée pendant 3 jours: la fièvre a été jugulée, la parasitémie diminuée n'a disparu que 7 jours après une *dose unique de* 25 *mgr. pyriméthamine* administrée 7 jours après le premier traitement. Le malade a reçu, par la suite, encore *deux doses de* 25 *mgr. pyriméthamine* 7 et 13 jours après. En observation pendant 6 mois n'a présenté ni fièvre, ni parasites — AoPo —.

3) Chez 5 malades: — S.M.[20]; E.G.[21]; D.S.[22]; H.H.[23]; S.J.[24] traités du 18[e] au 51[e] jour de l'infection avec 75 *mgr. pyriméthamine associée* à 1500 *mgr. chloroquine* (dose totale administrée en 3 jours), le parasites ont disparu du 4[e] au 6[e] jour chez 3 malades, chez les deux derniers — H.H.[23] et S.J.[24] — après respectivement 5 et 7 jours en association avec la plasmoquine: 0,02 gr. × 5 jours.

Les malades ayant quitté l'hôpital une semaine après la cure radicale, *on n'a pas signalé, jusqu'à ce jour, des éventuelles rechûtes par rapport au contrôle régional sur place.*

Conclusions générales

Des études effectuées sur 72 impaludés — en accord avec une « variante locale » du schéma des coordonées sous les auspices de l'O.M.S. — sur « l'action sporontocide sélective » de la pyriméthamine «seule», d'une part; d'autre part en association avec les 4 et 8 amino-quinoléïnes *dans la cure radicale du paludisme,* — apportent une série de faits d'application pratique non douteuse sur le rôle de la Chimiothérapie dans un programme d'éradication. Les résultats des recherches sur l'action de la pyriméthamine «en dose unique» contre les différents stades du cycle parasitaire, par rapport aux souches locales de *P. vivax, P. falciparum* et *P. malariae,* utilisées en impaludation thérapeutique viennent à l'appui des faits acquis par différents malariologistes.

A) *Infection à sporozoïtes de P. vivax.* Une « dose unique » de 25-50 mgr. de pyriméthamine, administrée avant l'impaludation de 6 malades à sporozoïtes et 2 à sang virulent n'exerce pas d'effet parasiticide contre: (a) le sporozoïte pendant son court circuit dans le sang; (b) ni contre les formes tissulaires préérythrocytaires. L'infection parasitaire a lieu: (1) dans 3 cas après une période de protection clino-prophylactique variable de 83, 65, et 54 jours; (2) de 255, 278 et 255 jours chez trois malades, qui avaient également reçu un traitement schizonto-gamétocide après 89, 59 et 47 jours, respectivement après l'administration de dose unique de 24 à 50 mgr. pyriméthamine (avant les impaludations répétées à sporozoïtes).

Les formes exoérythrocytaires tissulaire « tardives » de *vivax* sont nettement résistantes; l'apparition de rechûtes tardives chez 3 sur 11 porteurs de gamétocytes, respectivement 290, 211 et 272 jours après traitement à « dose unique» de pyriméthamine seule ou associés avec la chloroquine d'une part, 91, 43 et 85 jours, respectivement après une seconde dose unique en est la preuve évidente.

La pyriméthamine seule ou associée à la cloroquine, administrée à 11 porteurs de gamétocytes, dont le sang était sûrement infectant pour *A. atroparvus,* 1 à 24 heures avant le traitement, « exerce une action inhibitrice sur le cycle sporogonique en 100% des cas expérimentés ».

La dévitalisation « sporontocide » des gamétocytes, dans ces essais, est evidente jusqu'au 7e jour (dernier contrôle) après l'administration du produit, coïncidant, il est vrai avec une forte diminution de la gamétocytémie et l'absence d'oocystes dans les dissections des anophèles gorgés.

Les effets « schizontocides lents » de la dose unique de pyriméthamine, néanmoins constante — *contre les formes érythrocytaires* du parasite sont fortement activés et accélérés par association avec la chloroquine.

B) *Infection à P. falciparum* (1) L'action inhibitrice d'une dose unique de pyriméthamine seule ou associée avec la chloroquine sur le « cycle » sporogonique de 15 porteurs de gamétocytes, — 5 impaludés à sporozoïtes et 10 avec du sang virulent — et dont le sang était prouvé infectant pour *A. atroparvus* avant le traitement, « est confirmée en 100% de ces cas ».

La dévitalisation des gamétocytes est encore très nette 3 semaines après l'administration de la dose unique (dernière date de ces essais).

L'absence de rechûtes des gamétocytophores traités pendant une durée d'observation variable avec le malade de 30 à 329 jours est en accord et correspond à la rareté des rechûtes « de long terme » dans l'infection naturelle à *falciparum.* Le phénomène est nettement en contraste avec les particularités de résistance nette des formes exoérythrocytaires de *P. vivax.*

(2) *Clino-prophylaxie.* Une dose unique de 50 mgr. pyriméthamine, administrée à 3 malades avant l'impaludation à sporozoïtes n'a pas d'action

sur le sporozoïte et les formes préérythrocytaires. Le maladie clinique apparaît — 92, 186 et 141 jours — après l'administration du médicament.

C) Les résultats remarquables des essais sur l'association de la pyriméthamine avec 4 et 8 aminoquinoléïnes dans la cure radicale de 35 paralytiques impaludés — avec les souches locales de parasites, d'une part; ainsi que ceux de notre ancienne expérience 1948-1953 en chimiothérapie sur l'efficacité schizonto-gamétocide de l'association de la chloroquine avec un des 8 aminoquinoléïnes d'autre part, nous déterminent à affirmer qu'à l'état actuel des connaissances de chimiothérapie, on ne peut pas renoncer aux propositions faites dans le document de travail soumis aux débats du 7-e Comité d'Experts et que nous répétons ici — « d'associer l'action sporontocide de la pyriméthamine avec l'action schizonto-gamétocyde de 4 et 8 aminoquinoléïnes dans le but d'éliminer le parasite dans un programme d'éradication du paludisme ».

D) A l'état actuel des recherches, l'expérience roumaine des 10 dernières années — de recherches et d'action antipaludique — à l'aide des produits schizonto-gamétocides « combinés », ainsi que les recherches coordonées sur l'action sporontocide et gamétocide des « doses uniques » de — pyriméthamine, chloroquine, primaquine, chinocide — nous déterminent de soumettre aux débats du Com. d'Experts du Paludisme de l'O.M.S. une série de considérations, basées: (a) sur l'étude du comportement aux médicaments des souches locales de plasmodium, d'une part; (b) sur les possibilités locales d'administration et distribution systématique de ces produits anti-paludiques, d'autre part.

Quant au rôle des « schizonto-sporontocides », pendant les différentes phases du programme d'éradication, il n'y a pas de doute — nous semble-t-il qu'il serait nécessaire « dès la fin de la 2-e année de la « phase d'attaque » et par la suite tout particulièrement dans l'organisation de « la sourveillance active et passive » de procéder aux traitements à l'aide d'une médication « conjointement associée — d'un 4 aminoquinoléïne « chloroquine ou synonymes », avec un 8 aminoquinoléïne « primaquine ou synonymes »; en leur associant obligatoirement dans un schéma l'effet suppressif et sporontocide de la pyriméthamine.

Proposition de schéma.

Cas suspects: dose unique « 300 mgr. chloroquine avec 25 mgr. pyriméthamine ». Après confirmation du cas: traitement de 3 à 5 jours à la chloroquine 300 mgr. + plasmoquine 0,01 ou : primaquine 0,015 ou : plasmocide 0,02 gr suivi jusqu'à la fin de la saison épidémique de traitement suppressif hebdoma-

daire à 300 mgr. chloroquine ou synonymes et en y ajoutant toutes les 3 semaines une dose sporontocide de 25 mgr. pyriméthamine.

Contrôle et éventuellement répétition du traitement au printemps de l'année suivante.

AZIONE SPORONTOCIDA E CLINOPROFILATTICA DELLA PIRIMETAMINA

Lo studio dell'azione sporontocida selettiva della pirimetamina da sola, ed, in associazione con le 4 ed 8 aminochinoline, nella cura radicale della malaria effettuata su 72 malarizzati ha messo in evidenza una serie di fatti di indubbia applicazione pratica sul ruolo che può rivestire la chemioterapia in un programma di eradicazione. I risultati delle ricerche sull'azione della pirimetamina in dose unica contro i diversi stadi del ciclo parassitario dei ceppi locali di *P. vivax, P. falciparum* e *P. malariae* usati in malarioterapia confermano i fatti acquisiti ad opera di diversi malariologi.

Nell'infezione da sporozoiti di *P. vivax* una dose unica di 25-50 mgr. di pirimetamina non svolge azione parassiticida contro lo sporozoite nel suo breve soggiorno nel sangue, nè contro le forme tissulari preeritrocitarie. La pirimetamina sola o associata alla clorochina esercita azione inibitrice sul ciclo sporogonico nel 100% dei casi. Gli effetti schizontocidi della pirimetamina sono assai attivati ed accelerati dall'associazione con la clorochina.

Nell'infezione da *P. falciparum* l'azione inibitrice di una dose unica di pirimetamina, sola o associata alla clorochina, sul ciclo sporogonico è confermata nel 100% dei casi. La dose unica di 50 mgr. di pirimetamina non ha valore clinoprofilattico non avendo azione sugli sporozoiti e le forme preeritrocitiche.

Viene ancora confermata l'utilità, al fine della eliminazione del parassita in un programma di eradicazione dela malaria, di associare l'azione sporontocida della pirimetamina con quella schizonto-gametocida delle 4 ed 8 aminochinoline. E' infine proposto uno schema di trattamento.

THE SPORONTOCIDIC AND CLINOPROPHYLACTIC EFFECT OF PYRIMETHAMINE.

The study of the selective sporontocidic action of pyrimethamine alone or in combination with 4 or 8- aminoquinoline in the radical treatment of malaria, carried out on 72 malarial patients brought to light a series of facts of clear practical applicability to the role which chemotherapy might play in an eradication programme. The results of studies of the action of a single dose of pyrimethamine against the various stages of the parasitic cycle of local strains of *P. vivax, P. falciparum* and *P. malariae* used in malariotherapy confirm the facts collected by various malariologists.

In infections by sporozoites of *P. vivax* a single dose of 25-50 mg. of pyrimethamine shows no parasiticidal action against spell in the blood, nor against the pre-erythrocytic tissue forms. Pyrimethamine alone or combined with cloroquine exerted an inhibitory action on the sporogonic cycle in 100% of the cases. The schizontocidic effects of the pyrimethamine are fairly marked and accelerated by association with chloroquine.

With *P. falciparum* infections the inhibitory action of a single dose of pyrimethamine, alone or combined with chloroquine, on the sporogonic cycle was confirmed in 100% of the cases. A single dose of 50 mg. of pyrimethamine has no clinoprophylactic value, having no action on the sporozoites and pre-erythrocytic forms.

The usefulness, in a malaria eradication programme, of combining the sporontocidic action of pyrimethamine with the schizonto-gametocitic action of 4- or 8- aminoquinoline has been again confirmed. Finally a scheme of treatment is suggested.

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Contributions à la chimiothérapie du paludisme. Action sporotocide de la pyriméthamine associée à l'activité schizonto-gamétocide des 4 et 8 amino-quinoléïnes dans la cure radicale du paludisme.

Recherches coordinées sous les auspices de l'OMS. Juin 1958.

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DURATA DELLA SOPRAVVIVENZA DI *PLASMODIUM BERGHEI* NEL PIPISTRELLO INSETTIVORO ITALIANO *MINIOPTERUS SCHREIBERSII*

AUGUSTO CORRADETTI, FELICE VEROLINI e MARTA ROSTIROLLA (*)

Esperimenti di infezione di *Miniopterus schreibersii* con *Plasmodium berghei* e di subinoculazione del sangue del pipistrello a topolini hanno messo in evidenza che *P. berghei* non va incontro a sviluppo progressivo in questo ospite sperimentale e che la durata della sua sopravvivenza in esso è compresa tra 24 e meno di 72 ore.

Data la suscettibilità a *Plasmodium berghei* dimostrata da alcuni pipistrelli frugivori, abbiamo voluto verificare il comportamento di questo Plasmodio nel pipistrello insettivoro italiano *Miniopterus schreibersii*.

Come è noto, il più grande impedimento ad eseguire ricerche sperimentali su pipistrelli insettivori è costituito dal fatto che questi animali si mantengono in vita con estrema difficoltà e solo per qualche giorno dopo la cattura. Noi abbiamo somministrato loro latte in polvere molto diluito in acqua mediante una pipetta messa a contatto con la loro bocca varie volte nella giornata: versando qualche goccia di latte tra le labbra del pipistrello, questo spesso la deglutiva, e talvolta con vera avidità. Così abbiamo potuto far vivere un gruppo di *Miniopterus* per un numero di giorni sufficiente all'esecuzione degli esperimenti.

ESPERIMENTI

Gli esperimenti da noi eseguiti sono stati:

1) Inoculazione di *P. berghei* a pipistrelli della specie *Miniopterus schreibersii*.

2) Subinoculazione a topolini di sangue di pipistrelli inoculati con *P. berghei*.

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In questi esperimenti tutte le inoculazioni e subinoculazioni sono state eseguite mediante iniezione endoperitoneale di 2/10 di cc. di sangue citratato.

I risultati degli esperimenti sono stati i seguenti:

1) *Inoculazione di P. berghei a M. schreibersii.* Con sangue di ratto infetto da *P. berghei* sono stati inoculati 12 *M. schreibersii* che sopravissero rispettivamente: uno 1 giorno, tre 2 giorni, tre 3 giorni, due 4 giorni, due 5 giorni e uno 9 giorni.

Nessuno di questi pipistrelli presentò mai parassiti nel sangue.

2) *Subinoculazione a topolini di sangue di M. schreibersii con P. berghei.* Gli esperimenti di subinoculazione furono:

a) Quattro topolini furono inoculati col sangue di un *Miniopterus* 24 ore dopo che questo era stato inoculato con sangue infetto di *P. berghei.*

b) Da altri tre *Miniopterus* furono inoculati altri sei topolini (2 per ciascun *Miniopterus*) 72 ore dopo che i pipistrelli erano stati inoculati con sangue infetto da *P. berghei.*

c) Da altri tre *Miniopterus* si inocularono altri cinque topolini (2 + 2 + 1) con sangue prelevato 96 ore dopo l'inoculazione dei pipistrelli con sangue infetto da *P. berghei.*

d) Da altri tre *Miniopterus* si inocularono ancora cinque topolini (2 + 2 + 1) 120 ore dopo l'inoculazione con *P. berghei* degli animali datori.

e) Infine un topolino fu inoculato con sangue di un *Miniopterus* che era stato inoculato 9 giorni prima con sangue di ratto infetto da *P. berghei.*

Dei 21 topolini così inoculati si infettarono soltanto tre dei quattro a cui era stato iniettato sangue di *Miniopterus* inoculato 24 ore prima con sangue di ratto infetto da *P. berghei.* Tutti gli altri topolini subinoculati rimasero negativi.

I topolini che si sono infettati hanno sviluppato, come di regola, una infezione progressiva mortale: soltanto il tempo di incubazione è risultato un po' prolungato (7-8 giorni).

Discussione dei risultati

La nostra tecnica per mantenere in vita i pipistrelli non ha permesso una loro sopravvivenza talmente lunga da rivelare direttamente se i pipistrelli dopo un tempo d'incubazione più o meno lungo avrebbero contratto l'infezione da *P. berghei.* Nessun parassita è stato osservato negli esami di sangue nei giorni successivi all'inoculazione, ma il tempo di osservazione è stato troppo scarso (fino a un massimo di 5 giorni e per un solo pipistrello fino a 9 giorni dopo l'inoculazione) perchè questo solo dato possa ritenersi probativo.

Tuttavia gli esperimenti eseguiti sono riusciti a mettere in evidenza che l'ipotesi che *P. berghei* introdotto in *M. schreibersii* possa andare incontro ad

attiva moltiplicazione progressiva in questo ospite sperimentale è altamente improbabile.

Infatti nei giorni successivi all'inoculazione da *P. berghei* il sangue prelevato ai pipistrelli si è dimostrato a mano a mano sempre meno infettante per i topolini. Se i parassiti nel sangue dei pipistrelli fossero stati in progressivo accrescimento numerico, come avviene quando l'infezione è in incubazione, il sangue, a mano a mano che si procedeva nel tempo avrebbe dovuto contener sempre più parassiti e dimostrarsi di conseguenza sempre più infettante per i topolini.

I risultati degli esperimenti di subinoculazione dimostrano al contrario che il sangue dei pipistrelli non risultava infettante per i topolini già 72 ore dopo l'inoculazione dei parassiti nei pipistrelli datori. Poichè 24 ore dopo l'inoculazione dei pipistrelli il loro sangue risultava infettante si può determinare che il tempo di sopravvivenza di *P. berghei* in *M. schreibersii* è compreso tra 24 e meno di 72 ore.

LENGTH OF SURVIVAL OF « PLASMODIUM BERGHEI » IN THE ITALIAN INSECTIVOROUS BAT « MINIOPTERUS SCHREIBERSII »

Plasmodium berghei transmitted by blood inoculation to the bat *Miniopterus schreibersii* does not show any evidence of progressive multiplication in this experimental host. The experiments of subinoculation to mice demonstrate that *P. berghei* survives in the blood of *M. schreibersii* from 24 to less than 72 hours.

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THE INFLUENCE OF WAR ON THE DEVELOPMENT OF MALARIA CONTROL MEASURES

GORDON COVELL (*)

The development of antimalarial drugs and insecticides has been greatly stimulated by the exigencies of war. The need to find a substitute for quinine during World War I inspired the researches which resulted in the production of pamaquin, mepacrine and later chloroquine. The exigencies of World War II led to the production of proguanil and to the discovery of the insecticidal properties of DDT, while primaquine was developed to combat relapsing malaria in troops returning from the operations in Korea.

In a paper entitled *Malaria and War* published in 1943, the writer cited a number of instances in which the conduct of military operations had been profoundly affected by the incidence of malaria among the forces engaged (1). Examples quoted included the British expeditions to the Low Countries in 1747 and 1809, the Burma Wars of 1824 and 1826, the French operations in Madagascar in 1895, the British Ashanti expedition of 1896, the Macedonian and East African campaigns of the first world war and the Italian, South-east Asian and South-west Pacific campaigns of the second world war.

The influence of war on the development of antimalaria measures is perhaps less generally recognized, and it is thought that a brief review of the subject may be of some interest.

ANTIMALARIAL DRUGS

Prior to the outbreak of the first world war, the only drugs available for the prophylaxis and treatment of malaria were the alkaloids of cinchona, of which the most commonly used was quinine. This is an effective agent for terminating the clinical attack in the majority of malarial infections, and it is doubtful if any of the synthetic antimalarials now in use would have been

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developed had not Germany been deprived of all sources of quinine during the first world war. It was the necessity for finding an effective substitute for quinine which inspired the researches leading to the synthesis first of plasmochin (pamaquin) and later of atebrin (mepacrine).

Up to the time when this work was planned, all atempts to synthesize quinine had failed; but GUTTMANN and EHRLICH, many years earlier, had discovered that methylene blue stains and therefore presumably penetrates the malaria parasite and had observed some abatement of clinical symptoms in patients infected with *Plasmodium vivax* to whom the dye had been administered. With these experiments in mind, a team of German scientists embarked on a line of research destined to have far-reaching consequences. They inserted a basic side chain into the formula of methylene blue, and found that one of the resulting compounds had considerable activity against bird malaria. It seemed likely that the activity of the quinoline nucleus present in quinine might also be enhanced by the introduction of a similar side chain. This line of approach culminated in the synthesis of pamaquin, the first synthetic quinoline compound to exhibit effective action against human malaria parasites.

Pamaquin has a powerful destructive action on the gametocytes of *P. falciparum*, a property not possessed by any of the cinchona alkaloids; it also effects a marked reduction in the relapse rate of vivax malaria. It was not however a satisfactory substitute for quinine, because of its relatively high toxicity and the fact that it has little action on the asexual erythrocytic forms of *P. falciparum*. The Germans therefore embarked on further studies; they attached the basic side chain which had been evolved for pamaquin to other heterocyclic nuclei, and finally in 1930 produced the acridine compound mepacrine. This proved to have a powerful destructive action on the asexual erythrocytic forms of all species of human malaria parasite. It possesses all the antimalarial properties of quinine and against some strains of *P. falciparum* it is considerably more active. It has however the disadvantage of tinting the skin yellow and of producing in certain subjects undesirable side-effects.

When Indonesia fell to the Japanese in 1942, thus cutting off the supply of quinine to the Allies, steps were immediately taken to manufacture mepacrine in large quantities in both Great Britain and in the United States. Mepacrine prophylaxis was rigidly enforced among the Allied troops operating in the South-west Pacific and South-east Asia commands. This measure resulted in the effective control of malaria in both areas and played an important in achieving final victory.

The German programme of research on synthetic antimalarials had not ceased with the production of mepacrine. Investigations were continued with the object of developing a drug with equally powerful antimalarial properties

but without its disadvantages. Removal of the methoxy-bearing ring from the mepacrine molecule gave rise to resochin (chloroquine), a member of the 4-aminoquinoline group of compounds. Preliminary tests of this drug on a small series of patients in a German hospital were interpreted as indicating a considerable degree of toxicity, and further research was undertaken to counteract this supposed defect.

The second world war broke out while this work was still in progress and as field tests of the 4-aminoquinolines were as yet incomplete, the Germans adopted mepacrine as the standard antimalarial drug for their armies. After the occupation of France supplies of chloroquine were made available to the French authorities for tests in North Africa; when this area was occupied by the Allies, stocks of this drug fell into the hands of the Americans, who were already engaged on a gigantic research programme in which more than 14.000 compounds were eventually tested for antimalarial activity.

The Americans found that the early German tests of chloroquine had created an exaggerated impression of its toxicity; it proved in several respects to be more active than mepacrine and less likely to produce undesirable side-effects; in particular it did not cause any discoloration of the skin. It was not used to any great extent during the second world war but was soon afterwards adopted as the standard antimalarial drug for the United States Army.

During the latter half of the second world war, British chemists, adopting a new line of approach, evolved a biguanide compound, proguanil (paludrine), which proved to have remarkable antimalarial properties. This drug acts on the pre-erythrocytic form of *P. falciparum* and is therefore a causal prophylactic of infection due to this species of parasite; it is a good suppressive of all forms of malaria; it inhibits the late sporogonic forms of the parasite, so that mosquitoes feeding on a gametocyte carrier receiving therapeutic doses do not become infective; it has a lower toxicity than any other known antimalarial drug, and it can be marketed at a moderate price.

Primaquine, a member of the 8-aminoquinoline group of compounds evolved in the United States, is another drug which owes its origin to the exigencies of war. The researches leading up to its production were inspired by the urgent need for a drug which would prevent relapses of vivax malaria among troops returning from Korea without undue risk of toxicity. It is claimed that the maximum tolerated dose of primaquine is twice as great as that of pamaquin and that it is four times as active as the latter drug in the radical cure of vivax malaria.

Insecticides

Insecticides.

The greatest advance in the conduct of antimosquito measures in recent years has been the application of residual insecticides, of which the first was DDT. This compound had been synthesized as early as 1874, but its insecticidal powers were not discovered until 1939, when Swiss chemists were searching for a chemical which would destroy clothes moths. DDT was first used on a large scale in the early years of the war against the Colorado beetle, which threatened the Swiss potato crop at a time when military needs had made the conservation of all foodstuffs a matter of vital importance. The need for a synthetic insecticide had been enhanced by the shortage of pyrethrum, the bulk of which was then grown in Dalmatia and Japan, and in 1942 DDT was made available to the military authorities in Great Britain and in the United States. It was used with great effect for the prevention of typhus fever during the campaign in Italy, and later in the war it was employed on a large scale for the destruction of mosquitoes and other insect vectors of disease. Thus, although DDT was already in existence when war broke out, the researches which demonstrated its possibilities as an agent for malaria control were inspired directly by military considerations.

INFLUENZA DELLA GUERRA SULLO SVILUPPO DELLE MISURE DI CONTROLLO DELLA MALARIA

Le esigenze della guerra hanno esercitato una profonda influenza sullo sviluppo degli insetticidi ad azione residua e dei medicamenti che stanno alla base di ogni moderna campagna antimalarica. Il fatto che la Germania fu privata di tutte le sorgenti di chinino durante la prima guerra mondiale stimclò le ricerche che condussero alla produzione di pamaquin e mepacrina. La clorochina venne trovata come un ulteriore sviluppo lungo la stessa linea di ricerche, e le sue eccezionali proprietà quale medicamento antimalarico vennero dimostrate dagli americani nel corso del loro programma di ricerche in tempo di guerra. Gli studi che portarono alla produzione del proguanil iniziarono quando gli Alleati vennero a loro volta tagliati fuori da ogni fonte di chinino, per l'occupazione giapponese dell'Indonesia. La primachina venne prodotta nel tentativo di trovare un sostituto innocuo del pamaquin per la cura del personale che ritornava dal fronte coreano. Il DDT, il primo insetticida ad azione residua ed il più largamente usato, venne introdotto ed usato per la prima volta su vasta scala a seguito di necessità belliche.

Le due grandi guerre che devastarono il mondo nel 1914-18 e nel 1939-45 ebbero in tal modo almeno un effetto benefico, in quanto esse stimolarono lo sviluppo degli insetticidi di sintesi e dei medicamenti che riuscirono a togliere alla malaria la maggior parte del suo terrore e porteranno forse un giorno ad ottenere la sua eradicazione dalla terra intera. L'effetto immenso esercitato dalla guerra sul progresso della medicina e di molte altre branche scientifiche non è ancora completamente apprezzato. In nessun campo, comunque, il suo stimolo ha esercitato una influenza maggiore che in quello della lotta antimalarica.

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VENEZUELA AND THE WORLD MALARIA ERADICATION PROGRAM

ARNOLDO GABALDON, ARTURO L. BERTI and LACENIO GUERRERO (*)

Eradication of a disease is obtained by: (*a*) action on the soil or susceptible individual, as in the case of smallpox; (*b*) action on the seed or infected person, as happens with yaws; and (*c*) action against the sower or transmission factor as in malaria. In the first two situations the eradication program is basically a logistic problem. Malaria eradication has also to do with logistics, number of houses to be sprayed and insecticide required, but is fundamentally an epidemiological problem.

A tropical country, Venezuela, has after the Union of Soviet Socialist Republics and the United States of America, which are in the temperate zone, the largest area from which malaria has been eradicated. Proof of malaria eradication in Venezuela is based on a high level epidemiological surveillance service as shown in the reports on malaria eradication of the World Health Organization (1959) and Pan American Health Organization (1959).

Of the 21 republics in the Western Hemisphere, Venezuela was in proportion to her territory, the one most severely hit by malaria. The areas which were infected cover a surface of 600.000 square kilometers inhabited by 4.500.000 persons. The nation-wide capaign against malaria in this country, begun in 1945, was based on DDT indoor residual spraying, and had as its aim « the eradication of malaria from Venezuela » by « protecting all the houses of the malaria zone, even those of sparsely populated or mildly malarious districts » (GABALDON, 1949). It was also the first nation-wide campaign organized with such an objective (RUSSEL, 1952). The initial plan contemplated having the whole country malaria-free by 1955, after spraying, 100 per cent of the houses of the infected zone. This plan, however, was not feasible due to circumstances explained elsewhere (GABALDON and BERTI, 1955; GABALDON and GUERRERO, 1959).

The World Health Organization (1959) has presented a report on development of the malaria eradication programs of all countries engaged in such work. The Pan American Health Organization (1959) has also presented a report on the progress made in the Americas. The preparation of these reports

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TABLE 1.

Countries from which malaria has been eradicated with specific meausures grouped according to Regions of the World Health Organization.

(From World Health Organization, 1959)

African	American	Eastern Mediterranean	European	South-East Asia	Western Pacific
None	Barbados Chile Martinique Puerto Rico Tobago U.S.A.	Cyprus Gaza Strip	Byelorussia Corsica Italy Latvia Lithuania Moldavia Netherlands Ukraine	None	Singapore

is highly commendable and undoubtedly required a great effort. It seems of interest to review the Venezuelan data and to compare them with those presented by other countries engaged in this campaign.

Table 1 presents the countries from which malaria has been eradicated with specific measures. Outside of four islands (Barbados, Martinique, Puerto Rico, Tobago) no country of the tropical zone has accomplished total eradication of malaria from its territory. Furthermore, those countries of the European region had smaller infected areas than the area from which the disease has been eradicated in Venezuela. The two largest areas from which malaria has been totally eliminated are in the temperate zone, and the third largest is in the tropical zone. They are:

Union of Soviet Socialist Republics	6,898.000 sq.kms.
United States of America	2.257.809 sq.kms.
Venezuela	400,414 sq.kms.

In the Western Hemisphere the size of the areas now free of malaria is as follows:

United States of America	2,257,809 sq.kms.
Venezuela	400,414 sq.kms.
Brazil	115,887 sq.kms.
Chile	55,287 sq.kms.
Argentina	26,200 sq.kms.
Puerto Rico	8,865 sq.kms.
British Guiana	4,921 sq.kms.
Surinam	3,320 sq.kms.
Barbados	430 sq.kms.
Martinique	300 sq.kms.
Tobago	295 sq kms.
Guadeloupe	69 sq.kms.

These figures indicate that, outside of the United States of America which is in the temperate zone, the territories of all the other countries from which

malaria has been eradicated, which are mostly in the tropical zone, together form an area abouth half the size (215.574 sq. kms.) of the Venezuelan. This is of particular interest if it is recalled that Venezuela had, proportionate to its population and territory the areas most heavily infected with malaria in the Western Hemisphere.

It should be observed that the term « malaria eradication » as used by the World Health Organization and the Pan American Health Organization is rather lax. For instance, if it is true that the United States has achieved total elimination of the disease from most of its territory, it is also true that some infected foci are still present. This is accepted by American malariologists as shown by the following paragraph: « In 1957, as cases of malaria approached the vanishing poin, the evidence indicated that the goal of eradication of malaria in the United States has not yet been achieved although it was near at hand. While indigenous cases continue to occur, it was probable that most, though perhaps not all, resulted from recent reintroduction of the plasmodium into the mosquito population from foreign sources» (DUNN and BRODY 1959). In Venezuela by malaria eradication is understood the total absence of indigenous cases for three or more consecutive years as proved by adequate active and passive surveillance. The efficiency of this surveillance has been recently tested by a team sent at the request of the Venezuelan Government by the Pan American Health Organization. So far, the team has found absence of cases in the eradicated area and the presence of an appropriate organization of case detection which will not let infected persons pass unnoticed (BOSHELL, 1959).

PROGRAMS OF ERADICATION.

Eradication of a disease is obtained by: (a) action on the soil or susceptible individual, as in the case of smallpox; (b) action on the seed or infected person, as happens with yaws; and (c) action against the sower or transmission factor, as in malaria. In the first two situations the eradication program is basically a logistic problem. It is a question of knowing the size of the population to be vaccinated or to be treated, the number of workers to perform the job in a given time, and the quantity of vaccine or drug required. Malaria eradication has also to do with logistics, number of houses to be sprayed and insecticide required, but it is fundamentally an epidemiological problem. Conditions of epidemicity and endemicity change from region to region and they have an influence on the length of the campaign required to reach eradication (GABALDON, 1956). Vectors vary in strains and in species, from zone to zone, and accordingly malaria is responsive or partially refractory to the insecticide, which is the most important issue to determine at the beginning of a program (GABALDON, 1953). These concepts have been quite often forgotten by malariologists in recent times. It is essential, therefore, to have

a basic line of malaria prevalence before the program starts, and to measure it perioddically. Those programs failing to do this are based on the idea that malaria eradication is only a logistic problem, and at least some of them will sooner or later be involved in difficulties.

Table 2 presents the chronological progress of programs in some countries with areas from which malaria has been eradicated according to data published by the World Health Organization (1959). This table requires some remarks. Albania, Bulgaria and Surinam could not have started their simultaneous total coverage in 1958 because they have already areas from which malaria has been eradicated (see Table 3). What probably happened is that these countries started their programs by successive coverage of areas and reached total coverage in 1958. If Argentina had started her simultaneous total coverage in 1949, malaria should have been eradicated by now, unless there are unknown entomological problems. This is another misinformation because, as Table 3 shows, that country had more than 80 percent of her originally malarious territory still infected in 1958, and 55.6 percent of it was not regularly sprayed. The delay of malaria eradication in Greece, a country

TABLE 2.

Chronological progress of programs in some countries with areas where malaria has been eradicated.

(From World Health Organization, 1959)

Countries and Territories	Simultaneous total coverage		Successive coverage of malarious areas	
	Attack: date of beginning	Consolidation: date of beginning	Attack: date of beginning	Consolidation: expected date of beginning in last area
Albania	1958	—	—	—
Argentina	1949	—	—	—
Bulgaria	1958	—	—	—
Brazil	—	—	1959	—
British Guiana	—	—	1947	—
Guadeloupe	1957	—	—	—
Greece	1946	—	—	—
Portugal	—	—	1949	1959
Portuguese India	—	—	1948	1963
Spain	—	—	1947	1959
Surinam	1958	1962	—	—
Taiwan	1953	1957	—	—
URSS	—	—	1950	—
Venezuela	—	—	1950+	1960++

(+) Beginning of first sprayed area: 1945
(++) Year of first area without indigenous cases: 1949

TABLE 3.

Extent of the original malarious area and of the progress of the malaria eradication programs in 1958.

(From World Health Organization, 1959)

Regions and countries	Original Malarious Area		Areas with Malaria eradicated		Areas under Surveillance		Regularly Sprayed		Not Regularly Sprayed	
	Km²*	Pop.*	Percent of Km2.	Percent of Pop.	Percent of Km2.	Percent of Pop.	Percent of Km2.	Percent of Pop.	Percent of Km2.	Percent of Pop.
Amer can Regiôn:										
Argentina	270.0	2,289	9.7	11.2	8.5	32.4	26.2	23.5	55.6	32.9
British Guiana	215.8	500	2 3	86.0	-	-	9 2	13.4	88.5	0.6
Guadaloupe	1.1	214	6.1	16 3	66.2	60.3	27.7	23.4	-	-
Surinam	143.5	250	2.3	49 6	-	-	97 7	50.4	-	-
Venezuela	600.0	4,517	66.7	72.9	7.3	15.6	26.0	11.5	-	-
European Region:										
Albania	23.2	1,065**	-	55.1	-	-	-	44.9	-	-
Bulgaria	41.0	2,200	57.8	72.7	17.3	11 4	19.8	12.0	5.1	3.9
Greece	66.3	4,459	21.6	16.2	-	74 8	-	9.0	-	-
Portugal	-	1,860	-	94 6	-	-	-	5.4	-	-
Spain	-	8,000	-	97 0	-	2.6	-	0.4	-	-
U S S R	11,898.0	137,700	58.0	76.8	29.4	18.3	12.6	4.9	-	-
Yugoslavia	60.2	4,437	29.6	32.6	0.8	0.6	43 8	38.3	25.8	28.9
South East Asia Region:										
Portuguese India	2.0	132	15.0	14.4	-	-	40.0	43.2	45.0	42.4
Western Pacific Region:										
Taiwan	23.0	6,730	35.8	77.4	44.1	18.0	20.1	4.6	-	-

(*) In thousands

(**) In the original 1,300

with simultaneous total coverage since 1946, is mostly due to the known presence of physiologic resistance by the main vector species. Venezuela, which started her program in 1945, still has infected areas due to behaviouristic resistance of two of the responsible anophelines, *A. aquasalis* and *A. núñez-tovari* (GABALDON, 1953).

The Venezuelan experience indicated that the smallest political divisions, the Municipios, were the most appropriate units to be used in order to measure the eradicated areas. Only one indigenous case found in one Municipio was sufficient to consider it as still infected. If one Municipio after one or more years without indigenous cases showed one case which could be considered under this denomination, that Municipio was listed again as still infected. As the degree of endemicity and epidemicity was not the same all over the

infected area, some Municipios reached zero cases before neighbouring ones, so that they were like islands in the malarious sea that was Venezuela. Slowly as their number increased, the territory of the eradicated Municipios became larger and larger until it was possible to present a contiguous area of eradicated malaria of 180.000 sq. kms. (GABALDON and BERTI, 1954).

The progress of the malaria eradication program in Venezuela and its annual rate of increase has been as follows:

Year	Area (sq. kms.)	Percentage of increase
1951	131.954	
1952	156,938	18.9
1953	199,740	27.3
1954	248.701	24.5
1955	305,414	22.8
1956	361,045	18.2
1957	372,601	3.2
1958	400,414	7.5

It may be observed that in 1951 there was already a considerable area with three years without indigenous cases. The rate of increase of this area became smaller in the last years due to local difficulties in the work or to the presence of partially refractory malaria. The territory still infected in Venezuela is formed by 59 Municipios with an area of 155.874 sq. kms. and a population of 520.000 inhabitants. This does not mean that all this population lives in localities where malaria transmission still takes place. These Municipios had 7598 localities originally infected, in which there were in 1958 only 686 indigenous cases in 312 localities. It should be observed that of these localities 179 had only one case during the year, which may mean absence of transmission in them. Furthermore, 124 localities had two to ten cases and only nine localities had more than ten cases. These figures indicate that malaria is reaching the vanishing point in Venezuela and that if the work with drugs (pirimethamine and primaquine) is finally successful Venezuela may still be the first continental country in the tropical zone to become entirely free of malaria.

PROOF OF ERADICATION.

It was stated above that a malaria eradication program is not only a logistic problem but fundamentally an epidemiological problem. Nevertheless, the epidemiological information presented by countries engaged in such work, as shown in the reports of the World Health Organization (1959) and Pan

TABLE 4.

Epidemiological data of countries with areas from which malaria has been eradicated

(From World Health Organization, 1959)

Country and area6	Population	Slides Examined		Positive Slides		Parasites Found				Active Surveillance			Passive Surveillance		
		Number	Per 1000 inhabitants	Number	Per 100,000 inhabitants	P. vivax	P. falciparum	P. malariae	Mixed infections	Slides examined	Positive slides	Positive per 1,000 examined	Slides examined	Positive slides	Positive per 1,000 examined
Argentina															
Eradicated	256,000	10,128	39.6	122	47.7	122	0	0	0	9,304	122	13,1	-	-	-
Surveillance	743,000	12,996	17.5	25	3.4	25	0	0	0	12,347	14	1.1	-	-	-
Reg. Sprayed	537,000	13,625	25.4	660	122.9	617	36	3	4	10,412	383	36.8	-	-	-
Not Reg, Sprayed	753,000	3,993	5.3	300	39.8	300	0	0	0	3,993	110	27.5	-	-	-
Total	2,289,000	40,742	17.8	1,107	48.4	1,064	36	3	4	36,056	629	17.4	-	-	-
British Guiana															
Eradicated	430,000	1	0,002	0	0	-	-	-	-	-	-	-	-	-	-
Surveillance	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reg. Sprayed	67,000	1.520	22.7	51	76.1	8	23	20	0	-	-	-	-	-	-
Not Reg. Sprayed	3,000	-	-	-	0	-	-	-		-	-	-	90	12	133.3
Total	500,000	1,521	3.0	51	10.2	8	23	20	0	-	-	-	90	12	133.3
Venezuela															
Eradicated	3,294,000	145,654	44.2	113	3.4	100	6	6	1	133,889	58	0.4	11,601	55	5.0
Surveillance	703,000	69,614	99.0	50	7.1	46	2	2	0	63,110	38	0.6	6,488	12	1.2
Reg. Sprayed	520,000	269,436	518.1	975	187.5	901	60	4	10	264,306	888	3.4	4,657	85	18.3
Total	4,517.000	484,704	107.3	1,138	25.2	1,047	68	12	11	461,305	984	2.1	22,746	152	6.7
Yugoslavia															
Eradicated	1,431,000	5	0.003	4	0.3	4	0	0	0	0	-	-	- 0	-	-
Surveillance	25,000	334	13.4	0	0	-	-	-	-	0	-	-	0	-	-
Reg. Sprayed	1,700,000	26,875	15.8	1,151	67.7	1,137	12	1	0	678	26	38.3	21,903	1,117	51.0
Not Reg. Sprayea	1,281,000	27,214	21.2	43	3.4	42	0	1	1	8	3	375.0	30	2	66.7
Total	4,437,000	54,428	12,3	1,198	27.0	1,183	12	2	1	686	29	42.3	21,933	1,119	51.0

American Sanitary Organization (1959) is generally much smaller than that corresponding to spraying operations. Only four countries with areas from which malaria has been eradicated answered fully the questionnaire on which those reports are based. These data are presented in Table 4. The intensity of the search for malaria cases is shown by the number of slides examined per 1000 inhabitants. Venezuela occupies the first place with more than 10 percent of the population examined during the year. The other countries do not reach a level of 2 percent of the population examined during the year. This indicates the presence in Venezuela of a surveillance service far above the usual level in order to comply with the recommendations of the Expert Committee on Malaria of the World Health Organisation in its Sixth and Seventh Reports for eradicated areas where some spraying operations continue, as is the case of Venezuela in some portions of its malaria-free area, due to the triatomine problem.

In the eradicated areas the number of positive slides per 1000 inhabitants in Venezuela was 3.4, much lower than that of other countries. The figures of British Guiana and Yugoslavia for this area cannot be taken into consideration due to the small number of slides examined. It should be observed, however, that the index of positive slides per 1000 inhabitants in the area still infected and regularly sprayed in Venezuela, was 55 times the size of the same index in the eradicated area, which shows the tremendous difference of the number of cases of malaria found in both areas. This is not the case in other countries.

Table 4 also shows the results of the work of active and passive surveillance. In the eradicated area of Venezuela active surveillance showed in 1958 an index of 0.4 positive slides per 1000 slides examined, while passive surveillance showed an index of 5.0. This indicates that slides coming from medical dispensaries have a higher probability of demonstrating malaria parasites than those collected by active surveillance with rural visitors going from house to house in search of malaria cases. Such a difference is also shown by this index in the other areas of work. On the other hand, the difference between the absolute number of cases found by active and passive surveillance is small in the eradicated area and in the area with one or two years without indigenous cases, but is very large in the area still infected and regularly sprayed. This indicates that in the eradicated area the status of malaria may be fully shown by dispensary work, but in the area still infected an active search for cases is required under such conditions as exist in Venezuela. Table 4 shows also how much lower these indices are in Venezuela compared with those of other countries.

A classification of cases according to their origin is presented in Table 5. It is a pity that no tabulation for parasites was made, as this has a great epidemiological interest. In Venezuela, sporadic case means a relapse of

more than three years with confirmation of absence of transmission by intensive search of cases. It is believed that without this meaning some indigenous cases may be confused with sporadic cases. Probably due to this very strict definition the number of sporadic cases in Venezuela is much lower than those found in other countries. It is also observed that, according to

TABLE 5.

Classification of malaria cases in some countries with areas from where the disease has been eradicated

(From World Health Organization, 1959)

Countries and areas	Imported	Introducet	Sporadic	Induced	Indigenous
Argentina:					
Eradicated	—	122	—	—	—
Surveillance	—	24	1	—	—
Regular sprayed	79	156	97	3	325
Not regular sprayed	2	—	11	—	287
TOTAL	81	302	107	3	612
British Guiana:					
Eradicated	—	—	—	—	—
Surveillance	—	—	—	—	—
Regular sprayed	8	—	28	—	15
TOTAL	8	—	28	—	15
Venezuela:					
Eradicated	79	28	1	5	—
Surveillance	27	23	—	—	—
Regular sprayed	286	2	1	—	686
TOTAL	392	53	2	5	686
Yugoslavia:					
Eradicated	4	—	—	—	—
Surveillance	—	—	—	—	—
Regular sprayed	2	—	—	—	1149
Not regular sprayed	7	20	14	—	2
TOTAL	13	20	14	—	1151

the definition, an introduced case is secondary to an imported one. These cases are found in areas without insecticide or with delayed spraying. Therefore, no introduced cases are possible vithout the presence of imported ones, and their number should be smaller than that of the imported cases when a good surveillance exists. Table 5 shows the difference in reference to introduced cases between Venezuela and the other countries.

IL VENEZUELA ED IL PROGRAMMA MONDIALE DI ERADICAZIONE DELLA MALARIA

L'eradicazione di una malattia si ottiene mediante: *a*) azione sul terreno, o individuo suscettibile, come nel caso del vaiolo; *b*) azione sul seme, o persona infetta, come avviene con la Framboesia e *c*) azione contro il seminatore, o fattore di trasmissione, come nella malaria. Nei primi due casi il problema dell'eradicazione è essenzialmente logistico. L'eradicazione della malaria ha pure a che fare con la logistica — numero delle case da irrorare ed insetticida necessario — ma è fondamentalmente un problema epidemiologico.

Un paese tropicale, il Venezuela, ha dopo l'U.R.S.S. e gli U.S.A., che sono in zone temperate, la più grande area da cui la malaria sia stata eradicata. La prova dell'eradicazione della malaria nel Venezuela è basata su un ottimo servizio di controllo epidemiologico come dimostrato nelle relazioni sulla eradicazione della malaria dell'O.M.S. (1959) e dell'Organizzazione Sanitaria Pan-Americana (1959).

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A NEW SUB-SPECIES OF *PLASMODIUM CYNOMOLGI*

P. C. C. GARNHAM (*)

1) A new sub-species of *Plasmodium cynomolgi - bastianelli* is described from a *Macaca irus* monkey from Malaya.
2) Minor differences exist in the blood and sporogonic stages.
3) Clear cut differences in the morphology and duration of pre-erythrocytic schizogony were demonstrated.
4) There is an absence of cross immunity between the type and the sub-species.

The classification of malaria parasites into different species was attempted soon after LAVERAN's original discovery of the organism in the bloodstream of man. The determination of the nature of their sporogonic cycles in the mosquito host confirmed the plurality theory of species which is of course completely accepted today. It was BASTIANELLI in conjunction with GRASSI who carried out this latter research and thus demonstrated the importance of the sporogonic stages in the taxonomy of the group. Malaria parasites were discovered in monkeys a little later, but their classification into species was hindered for a long time because our knowledge regarding them was confined to the stages in the blood, and it was many years before BASTIANELLI's criteria of sporogonic development could be applied.

The greatest complexity in simian malaria is found in the East Indies and the Far East, where four principal types are now distinguished: *Plasmodium knowlesi* (with a quotidian cycle), *P. cynomolgi* (with a tertian cycle) and *P. inui* (with a quartan cycle) as true plasmodia and *Hepatocystis* (= *Plasmodium*) *semnopitheci* (with only gametocytes in the blood). The type represented by *P. cynomolgi* occurs in at least two forms: the well known parasite described originally by MAYER (1907) in a *Macara irus* coming from Java, and subsequently isolated and maintained in the Malaria Institute of India. It was redescribed in detail by MULLIGAN (1935). A less known form was found in Formosa by

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INOKI *et al* (1942) in *Macaca cyclopis* and named *Plasmodium inui* var. *cyclopis*. It was said to differ from *P. inui* var. *cynomolgi* in its greater virulence to rhesus monkeys. This parasite has a tertian periodicity, and produces Schüffner's dots in the corpuscle; its correct designation is *P.cynomolgi cyclopis*.

This paper describes a third form of *P. cynomolgi* on which I have been working for the past three years, and which I propose to name *Plasmodium cynomolgi bastianellii* s. sp. nov. in honour of the last of the original band of Italian malariologists. The sub-species is described below.

ORIGIN OF STRAIN

In July, 1956, Dr. J.F.B. EDESON of the Institute of Medical Research, Kuala Lumpur, Malaya, sent some malaria infected kra monkeyes (*Macaca irus*) to Dr. F. HAWKING (HAWKING *et al* 1957) in London. One of these monkeys (Malayan number 44) was said to have shown a scanty mixed infection of *P. cynomolgi* and *P. inui*, but subsequent transfers demonstrated that the latter species had probably been mistaken for *P. knowlesi*. A rhesus monkey was inoculated with its blood, and when parasites appeared a second blood passage was made into another rhesus. On the latter becoming infected, mosquitoes were allowed to feed on it several times, and the sporozoites which later appeared in their salivary glands were injected into a further rhesus monkey (HAWKING *et al's* number 288: my number M. 186); pre-erythrocytic schizonts resembling *P. cynomolgi* were found a week later in the liver, and trophozoites of the same species, contaminated with *P. knowlesi*, subsequently appeared in the blood. It is evident that HAWKING *et al* had obtained a double infection with the two species in their mosquitoes, which later infected monkey M. 186. The intensity of the *P. knowlesi* infection was dampened by the administration of small doses of mepacrine on numerous occasions and the monkey was given to me six months after its first infection. I splenectomised it on September 6th, 1957 and 10 days later, when gametocytes of the « *cynomolgi* » type were numerous, I allowed a batch of *Anopheles aztecus* to feed on it. These mosquitoes became heavily infected, and sporozoites were inoculated intravenously into another rhesus monkey (M. 193). A pure line of the « *cynomolgi* » was thus established, and the following passages were made:

M. 193 sporozoite induced infection from M. 186.

M. 197 » » » » M. 193

M. 162 (*Cebus*), M. 167 (*irus*), M. 198 & M. 199 (*Papio*) sporozoite induced infections from M. 197.

M. 200 sporozoite induced infection from M. 198.

(All monkeys were rhesus unless otherwise stated).

Stages in the blood

The asexual development of the parasite in erythrocytes resembles that of the type species, in that amoeboid forms are produced which give rise to schizonts containing 12-18 merozoites, and which cause enlargement and Schüffner's stippling of the host cell. Multiple invasion of the latter is common, and grains of pigment are present, light brown in colour, later darkening as agglomeration takes place. Minor and inconstant differences were noted as follows: —

The mature schizont does not always fill the erythrocyte, which is sometimes enormously enlarged. A prominent residual body is often present. Vacuolation of the cytoplasm of the large ring forms is frequent. Schüffner's dots tend to stain less intensely than in the type species.

The duration of the schizogonic cycle in the blood is 48 hours, but instead of maturation of the schizonts occurring in the early hours of alternate mornings (as it does fairly constantly in the type), it takes place just before midday, with a rather precise synchronicity. A further point of difference is the manner in which the blood infection terminates in a definite crisis after about 7 to 9 days, with a great drop in parasitaemia and the production of abnormal forms. Gametocytes are equally affected by the crisis.

The sexual development in the blood stream appears to be indistinguishable in the two strains.

Stages in the mosquito

Anopheles maculipennis atroparvus, A. aztecus and *A. stephensi* are easily infected with this parasite. At 27° C, sporogony proceeds as follows:

On the third day, the oocyst is about 12μ in diameter, and on the fourth, about 20μ. Pigment granules, brownish yellow to black in colour, are visible on these days in longer or shorter rows. By the seventh day, the oocyst reaches a size of 45μ and sporozoite formation has started, while on the eighth day, the oocyst is mature and the salivary glands become invaded by sporozoites. The average length of the sporozoite is 15μ. The duration of sporogony is thus seen to be about a day shorter than in the type form, which at 27° C is 9 days.

Pre-erythrocytic schizogony

My attention was first drawn to the unusual behaviour of this strain by the brevity of the prepatent period. After a heavy sporozoite infection with *P. cynomolgi,* the blood invariably becomes invaded on the eighth day (Bray,

1957); with the new parasite the period was found to be seven days. I have shown elsewhere (GARNHAM, 1959) that this character and the general nature of pre-erythrocytic schizogony are of great taxonomic value in the genus *Plasmodium*. A detailed study was therefore made of the stages in the liver of this new parasite as compared with similar stages of the type species, particularly on the sixth and seventh days.

On the sixth day, the sub-species produces pre-erythrocytic schizonts up to 24μ in diameter with prominent cytoplasmic clumps, and nuclei sometimes in the bar form. The cytolasm may contain small vacuoles only (Fig. 1). In the type form at 6 days (Fig. 2) the schizont is almost the same size and shows similar characters except that large vacuoles may have started to form. An important difference is the earlier appearance of desoxyribonucleic acid in the nuclei of the schizonts of the sub-species, as shown by the positive Feulgen reaction. This reaction is negative, even on the seventh day, in the nuclei of the type species.

The major morphological differences are seen on the seventh day. In the sub-species, the schizonts begin to mature and measure only 30μ in diameter (Fig. 3); vacuoles are absent. In the type species on the other hand, the schizonts are still a day off maturity, but measure about 34μ and may often contain prominent vacuoles (Fig. 4), as was noted in our original work (SHORTT & GARNHAM, 1948). It should be noted that the main bursting of the schizonts takes place in both parasites nearly a day later than at the minimum times. At actual maturity of the sub-species, the schizonts are still less than 35μ in diameter, and show an indefinite patterning of the merozoites; moreover the peripheral nuclei are elongated, and lie in parallel rows at right angles to the surface (Fig. 5). This appearance is often seen in *P. inui*, but I have never observed it in the type form of *P. cynomolgi*.

CROSS IMMUNITY TESTS

Although the possession or absence of cross immunity between two strains of an organism is no absolute guide to their identity or distinction, the test provides a useful indication; if, for instance, cross immunity were shown to exist between two strains, it would probably be correct to assume (in the realm of malaria parasites) that they were not different species. Observations were accordingly made on two monkeys, immunized against the type species (the so-called « Rockefeller » strain, originally obtained from the Malaria Institute of India) as follows:

M. 146 (rhesus monkey) had been infected by sporozoites of the type species on 21/6/54 and a typical infection followed; the spleen was removed on 2/4/58 and a relapse occurred, though parasitaemia disappeared three weeks

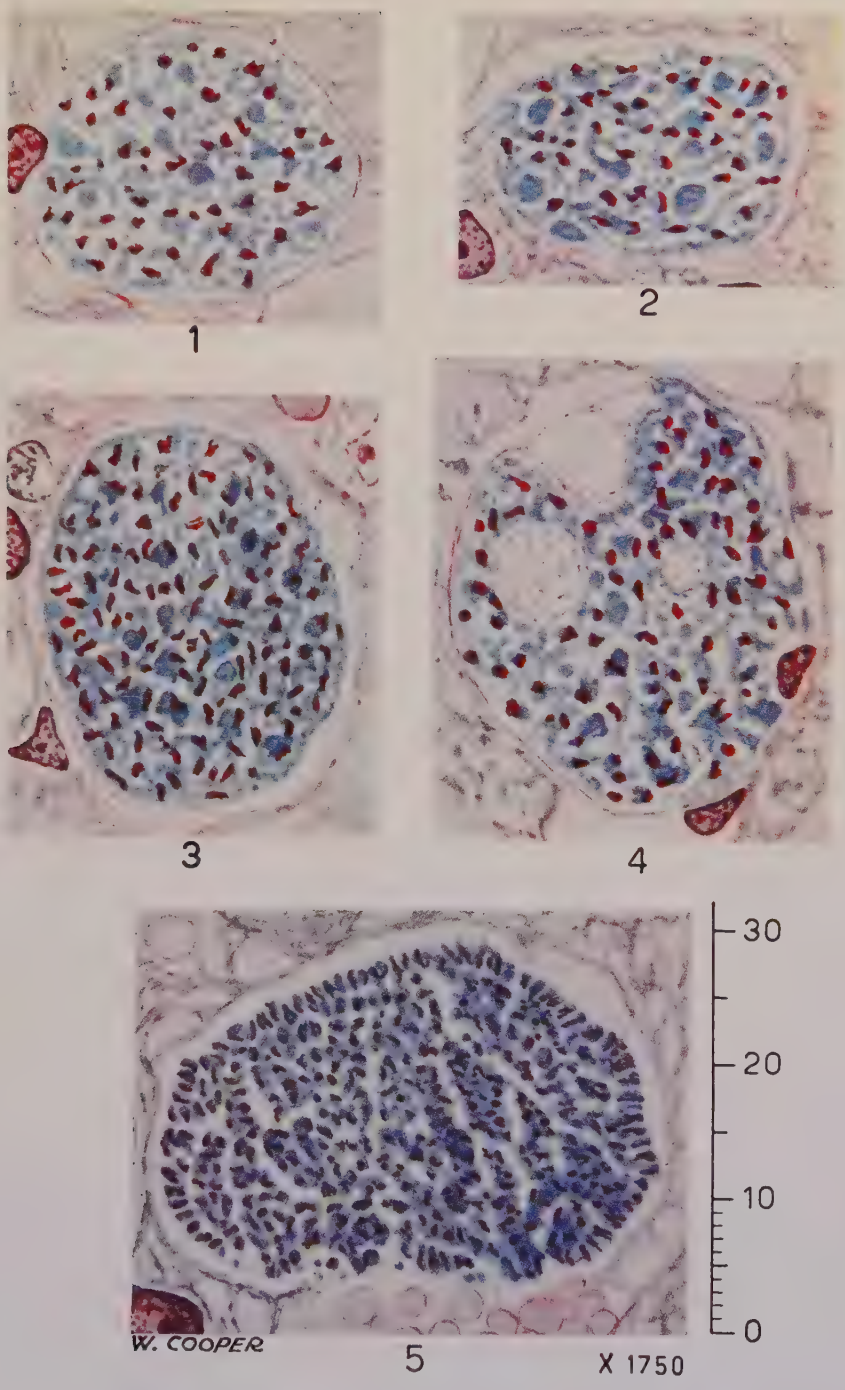

Fig. 1 - Section of liver of monkey containing a 6th day pre-erythrocytic schizont of *P. cynomolgi bastianellii.* Fig. 2 - Section of liver of monkey containing a 6th day pre-erythrocytic schizont of *P. cynomolgi.* Fig. 3 - Section of liver of monkey containing a 7th day pre-erythrocytic schizont of *P. cynomolgi bastianellii* approaching maturity. Fig. 4 - Section of liver of monkey containing a 7th day pre-erythrocytic schizont of *P. cynomolgi.* Fig. 5 - Section of liver of monkey showing a mature pre-erythrocytic schizont of *P. cynomolgi bastianellii.*

(All sections were prepared with Carnoy fixed material and stained by the Giemsa Colophonium method).

later and the blood remained clear of parasites. On 28/10/58, the monkey received intraperitoneally 1 cc of blood containing the new strain (from M. 200); three days later parasites were found in thick films of its blood and the infection reached a crisis on 7/11/58 when the density of parasitaemia reached 500 per 10,000 erythrocytes. The parasites then rapidly declined in numbers.

M. 96 (rhesus monkey) was infected by blood containing the type form of *P. cynomolgi* on 7/4/52, and an ordinary infection ensued. It was subsequently challenged with infected blood on 14/5/53, 24/8/53 and 2/2/55, and with sporozoites on 28/7/54, and showed few or no parasites after these inoculations of the type form. On 28/10/58 it received intraperitoneally 1 cc of blood containing the new strain of parasite (from M. 200); a day later parasites were found in the thick film and a crisis, with 450 parasites per 10,000 corpuscles, was reached on 5/11/58, after which the blood soon became parasite free.

These two monkeys were apparently immune to the type form of *P. cynomolgi,* yet they easily became infected with the new parasite, and a parasitaemia of moderate intensity followed in each instance. The absence of cross immunity offers further confirmation that the two parasites are different.

Discussion

Apart from a few minor differences, the blood and sporogonic stages of the two parasites resemble each other closely, and it is clear that the new strain belongs to the *cynomolgi-vivax* group. Clear cut differences however are demonstrable in the nature of pre-erythrocytic schizogony, while cross immunity tests provide additional evidence that a sub-specific difference at least is present.

It should be noted that the original isolation was made from a kra monkey in which *P. knowlesi* was also present; this parasite contaminated the first mosquito passage, and the question arises as to whether hybridisation could have occurred. From our knowledge of other species, this seems to be unlikely, but the possibility cannot be entirely discounted.

Exposure of M. 186 to mepacrine might also be thought to have caused a change in the morphology of the parasite, but again we have no evidence that malaria parasites can be permanently affected by the action of drugs.

The characters of the sub-species are remarkably constant and were seen in several species of monkey.

UNA NUOVA SOTTOSPECIE DI *PLASMODIUM CYNOMOLGI*

Nel corso di osservazioni su un ceppo di *P. cynomolgi* della Malesia recentemente isolato, fu notato che il periodo di incubazione era regolarmente un giorno più breve che nella specie tipo.

Sono stati studiati in dettaglio sette casi di infezione mediante sporozoiti nel rhesus ed in altre scimmie. Si è visto che la schizogonia nel sangue raggiunge il suo massimo verso il mezzogiorno a giorni alterni, invece di quasi 12 ore più presto, mentre l'infezione giunge a una crisi alla fine della prima settimana. La sporogonia nella zanzara è di un giorno più breve che nella specie tipo. La durata della schizogonia pre-eritrocitaria è di 7 giorni, invece di 8 come nella specie tipo, ed inoltre gli schizonti pre-eritricitici sono più piccoli e non contengono grandi vacuoli; nei nuclei di questi schizonti compare precocemente acido desossiribonucleico. Scimmie immuni al ceppo tipo sono risultate suscettibili al nuovo ceppo. Per questi motivi il parassita viene considerato una sotto-specie di *Plasmodium cynomolgi* e gli viene assegnato il nome di s.sp. *bastianellii* in onore del defunto Prof. G. Bastianelli.

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FIFTEEN YEARS EXPERIENCE IN MALARIA ERADICATION IN BRITISH GUIANA. MAINTENANCE POLICIES AND RESIDUAL PROBLEMS

G. Giglioli (*)

An outline is given of the Malaria campaign in British Guiana leading to the eradication of both hyperendemic malaria and its carrier *A. darlingi* from the coastlands, which sustain 90 per cent of the territory's population.

A description is given of maintenance techniques and of the nature and entity of residual malaria problems; these involve a population of only 20,000 scattered over vast areas of the remote interior. Plans are outlined for the final eradication of malaria from these areas by means of Pinotti's medicated salt technique.

British Guiana lies on the North East coast of South America between the 1st and 8th degrees of Northern Latitude and between the 56th and 62nd degrees of Western Longitude. It is therefore a continental territory situated within the equatorial belt; it covers an area of 83,000 square miles.

This large area has a population of 518,000, nine tenths of which are settled on a narrow strip extending for approximately 200 miles along the Atlantic coast between the mouths of the Pomeroon and Corentyne rivers This strip varies in depth from 5 to 10 miles, including the extensive cultivation of sugar cane and rice which constitute the basic local agricultural crops.

The coastal population aggregates approximately 450,000 people mainly of East Indian, African and mixed descent, with much smaller numbers of Chinese and Europeans. The remaining 1/10 of the territory's population is scattered over the interior, along the tidal reaches of the larger rivers, on the Pakaraima Plateau and on the Rupununi Savannahs. The Eastern half of the territory's interior, towards the Surinam border, is uninhabited.

Most of the interior is covered with equatorial rain forest. Savannahs

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occur only in parts of the Pakaraima Plateau and further south between the Rupununi and Takatu rivers. These savannah merge with the Gran Sabana of Venezuela and the Rio Branco Savannah of Brazil. The population of the upper reaches of the rivers and of the Savannahs is mainly of Amerindian race, still in a more or less primitive state of civilisation.

Communications with the interior are by river; the more remote areas are only accessible by plane and from the landing fields further travel is by canoe, by horse or on foot.

Indians prefer to live in small isolated settlements of one to three or four houses, scattered over wide areas; they cultivate farms in the forest, shifting to virgin sites every two or three years as soon as the soil becomes exhausted. These farms are frequently situated at long distances from the settlements so that the whole Indian family passes a considerable portion of its existence camping out under temporary palm-leaf shelters when clearing the forest for new fields, planting or reaping; the same happens during their frequent hunting and fishing expeditions.

In British Guiana malaria was transmitted by *A. darlingi,* an efficient carrier with highly selective anthropophilic and endophilic biting habits. *A. aquasalis,* a potential carrier, is very numerous along the coast but it is entirely fixed by the large cattle population. Only on the lower tidal reaches of the rivers in the North West District of British Guiana, bordering on the Delta of the Orinoco, *A. darlingi* was not found, *A. aquasalis* being the vector and maintaining a mildly endemic malaria; the absence of livestock in this forested area causes this mosquito to pay considerably more attention to man and to enter houses more frequently than elsewhere.

A. albitarsis, another potential malaria carrier, is extremely abundant in the well stocked savannahs of the coastland and of the interior, but has never been associated with malaria transmission.

A. nunes tovari, a proven exophilic carrier in Western Venezuela, has been reported from the Demerara river area but only in very low densities and with no association with malaria transmission.

A. bellator, a bromelia breeder, is a well known malaria carrier in the cocoa plantations of Trinidad. In British Guiana it has been found only West of the Essequibo on the upper reaches of the rivers in the Nort West District, and on the upper Potaro in the Pakaraima Plateau. In most instances it is a forest mosquito of low density with no malaria association. Recently however, CHARLES (1959) has incriminated this mosquito for a persistent focus of endemic malaria at Koriabo on the upper Barima river, in an area of the North West District where *A. darlingi* has been eliminated and *A. acquasalis* does not occur.

The malaria problem of the British Guiana coastlands was probably one of the gravest in this hemisphere. The land is mostly below high tide level,

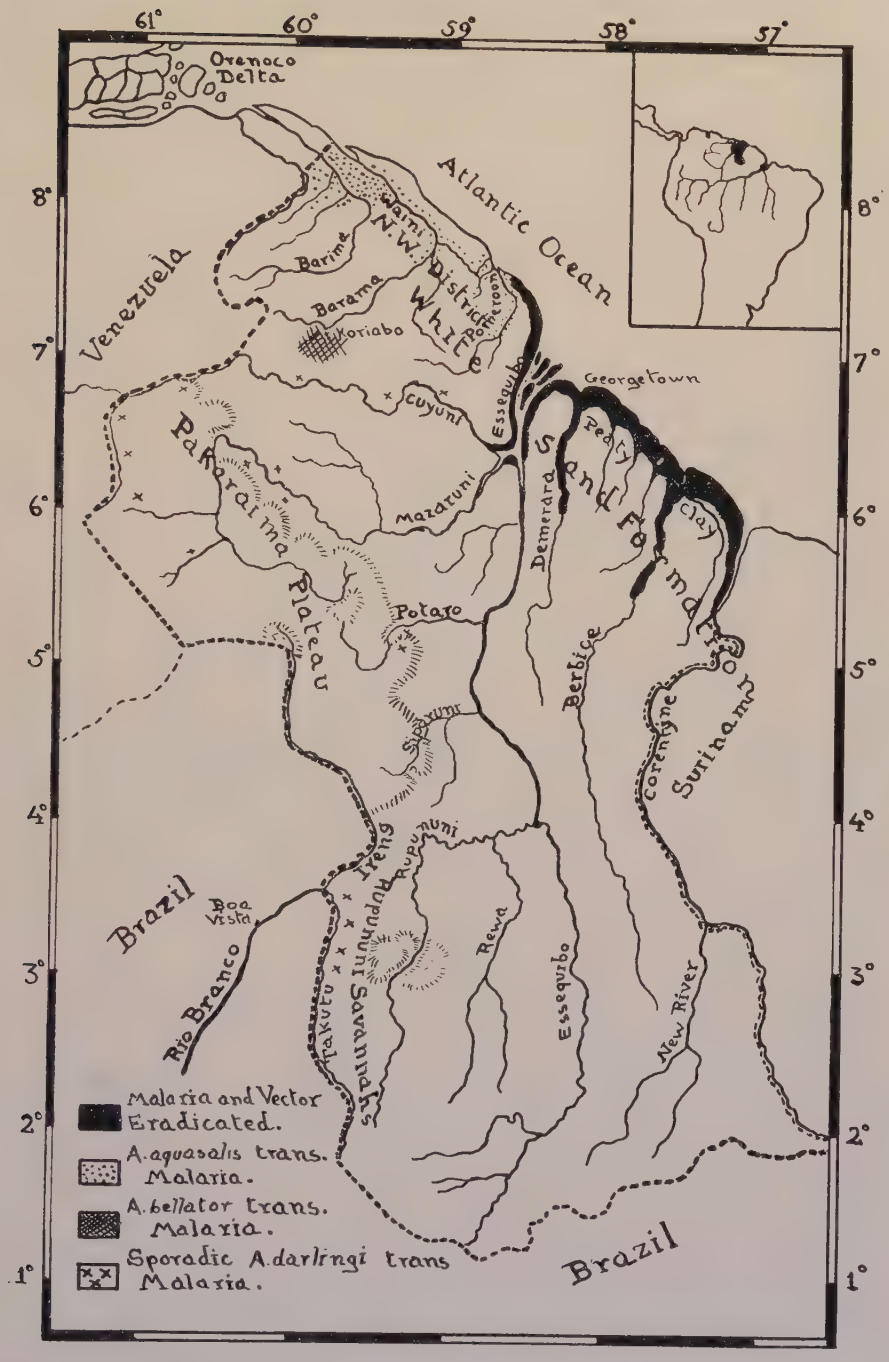

Fig. 1. — British Guiana: Showing areas from which both Malaria and *A. darlingi* have been eradicated (90% of population) and areas of the Interior with residual Malaria Problems in October 1959 (4% of population).

and protected from flooding from the sea and from interior by dykes as practised in the Polders of Holland. The rainfall approximates 100 inches per annum; drainage is provided by an elaborate system of canals, which discharge through sluice gates at low tide (8 hours out of 24) or through powerful pumping stations. An immense net-work of canals provides for irrigation and internal navigation in the cultivated areas. As an average there are 15 miles of canal for every square mile of cultivation. These canals have a constant level all the year round and, with the shelter provided by tall sugar cane, provide ideal breeding grounds at all seasons. Under such conditions, the production of *A. darlingi* on the coast used to be continuous and it could be found in large numbers in rural houses at all seasons. The two crops on which the territory's economy is based are sugar cane and rice, both requiring enormous amounts of water and extensive periodical flooding. Thus agricultural development, implying extension of sea defenses, drainage and irrigation, inevitably and tragically also implied an extension of gravely endemic malaria.

Up to 1917 the population maintained itself mainly through immigration from India and the West Indian islands. As recently as 1945 the vital index in some of the coastal districts oscillated around 100 and in some years it fell below that figure. The natural increment of the population was maintained by the population of Georgetown and of the eastern coastlands; East of the Berbice river, in fact, malaria was mild with periodical epidemic waves during years of high rainfall. This was due to the lack of adequate sea defenses and frequent sea flooding around the inhabited areas.

D.D.T. got to British Guiana early in 1945 at a time when very detailed data on the epidemiology of malaria and the habits of its local vector had already been acquired. This enabled us to apply the new residual insecticide technique appropriately and economically, restricting our whole effort on the highly domestic *A. darlingi* adults. Every rural house including those situated in city suburbs was sprayed with D.D.T., with complete « blanket » coverage. As early as August 1948 we were able to report (Giglioli 1948):

« At the time of writing 325,000 people, representing 90% of the Colony's population are already enjoying the many benefits of DDT protection; all the evidence indicates that *both A. darlingi and Ae. aegypti have been eradicated from the treated areas*, some 200 miles of coastlands and estuary banks ».

« The eradication of this very dangerous malaria carrier has been achieved by DDT used exclusively as a residual spray applied to the interior of houses, completely ignoring all phases of hydrological control. These results having been obtained on the Coastlands of British Guiana are all the more significant as a more hopelessly difficult hydrological situation could hardly be conceived ».

We have described very briefly the peculiar and mostly man-made characteristics of the inhabited and cultivated coastal fringe: a narrow ribbon,

usually less than 10 miles broad, running for some 200 miles along the North-Eastern coast of the South American continent, narrowly sandwiched between the Atlantic Ocean and the immense rain forests of the interior. With both malaria and *A. darlingi* eradicated from the coastal strip our new problem was the maintenance of our conquests. At first the indefinite continuation of costly periodical spraying operations throughout the area appeared inevitable. Eradication of the vector having been achieved, we were more pre-occupied with possible re-introduction of *A. darlingi* than with that of *Plasmodium* carriers, as the latter would have no public health significance in the absence of a suitable vector.

In British Guiana *A. darlingi* has always been found to be strictly endophilic and anthropophilic; this however does not constitute the constant pattern of behaviour of this species, as has been demonstrated in central Brazil, in some parts of Amazonia and in a restricted area in Venezuela. We can not exclude that this species still exists independently of man in our vast forests in the interior, thus the possibility of re-invasion of our coastlands from interior must be entertained.

The geographical distribution of *A. darlingi* is very wide, covering most of tropical America east of the Andes. It is probable that this species originated on the central plateau of Brazil. In this area *A. darlingi* appears to be eclectic in its biting habits, attacking both animals and man, within the house and in the open. Towards the periphery of its present-day area of distribution along the North and Eastern coast of South America, on the contrary, it is strictly anthropophilic and endophilic. This specialisation was presumably acquired by following man, along the forced lines of communication, through the impervious equatorial forest, constituted by the rivers, in areas devoid of domestic livestock and with a relative scarcity of wild animals.

In British Guiana, a country conspicuously deficient in lime, with acid soils, ground waters tend to be acid, with a remarkable pH range (3.5 to 6.4). *A. darlingi* presents selective breeding habits, preferring waters which are only slightly acid or near neutral. Thus large surfaces of waters draining from certain types of geological formation and soils are quite unsuitable for the production of this dangerous mosquito. (Giglioli 1951 a & b).

The fertile coastal strip of British Guiana is formed by an alluvial clay deposit of marine origin, which is of moderate acidity; salt and acid have been reduced by drainage, constant cultivation and irrigation. Further inland, beyond the inhabited and cultivated fringe there are extensive peaty swamps, generally used as catchment areas for the conservation of water for irrigation; these waters have a characteristic brown colour and are very acid. Still further inland, and running through the whole width of the territory and on through Dutch Guiana, is another characteristic geological formation consisting of a thick deposit of pure white quartz sand, originally a submarine

plain, but now elevated and dissected by erosion into a system of low, flat topped sand hills. This formation carries a special type of forest and also drains waters which are deeply stained with vegetable matter and very acid. Further inland on the more ancient continental formations, soils are mainly constituted by red lateritic loams, and the waters which drain from them are colourless, clear, less acid and better suited to *A. darlingi* breeding.

Thus the populated coastal strip from which Malaria and *A. darlingi* have been eradicated is separated from any possible *A. darlingi* haunts in the interior by two invisible barriers:

(1) The coastal peaty clays, locally known as « Pegasse » clays; and

(2) The White Sand formation.

The only avenues through which *A. darlingi* could again reach the coast are the narrow alluvial banks of the rivers and smaller water courses which traverse both these formations.

Since 1952 we have closed down spraying operations throughout the whole coastal strip, present population 450,000. We have integrated the natural barriers formed by the « pegasse clays » and the White Sand Formation by continuing to spray — on 18 month cycles — the relatively small number ot houses situated along the rivers and water courses where they traverse the barriers. Thus for the past 8 years we have treated the densely inhabited coastal strip as if it were an island and we have successfully and economically maintained within this area both the eradication of malaria and of *A. darlingi* its local carrier.

Our system of surveillance throughout the medically well organised coastal strip has been based on notification of supsicious fever cases and on entomological surveys.

In the Interior eradication appears to have been achieved in all the more densely and permanently inhabited areas along the tidal reaches of the Essequibo, Berbice and Corentyne Rivers. These districts aggregate a population of approximately 30,000. However, these are all forest areas where surveillance is difficult and expensive; spraying operations have therefore been continued on an annual basis. It is not unlikely that this practice may be superfluous, but an excess of prudence appears advisable; the possibility of insecticide resistance developing in *A. darlingi* can be discarded and annual spraying operations are much cheaper, simpler and more reliable than year-round surveillance.

The more remote parts of the country, aggregate a population of approximately 20,000 widely scattered over the western part of the territory. All inhabited areas are covered by DDT spraying on an annual cycle but in consequence of difficult communications, supervision has not always been as effective as it should be and localised lacunae or inappropriate timing of spraying have been found from time to time. Malaria incidence has been

reduced to an extremely low level but eradication was never completely achieved or claimed. The reasons for these incomplete results can be listed as follows:

1) The Amerindian population lives in permanent houses or settlements which are subject to DDT spraying but they spend a very considerable part of their existence in the forest, camping out under temporary shelters when cultivating their farms, or when on fishing or hunting expeditions. *A. darlingi* has been found in these houseless, intermittently inhabited localities, on farms or in habitual camping sites along well beaten trails or waterways. Under such conditions residual methods are not applicable and transmission continues on a reduced scale.

2) About 7,000 Amerindians live close to the frontier with Brazil along the Irens and Takatu rivers; they move freely across the border. No control measures have been applied so far in the Rio Branco Federal Territory of Brazil beyond the immediate neighbourhood of the capital Boa Vista. Thus infection is inevitably imported. Eradication cannot be finalised without International co-ordination.

3) In the North West District, on the lower tidal reaches of the many rivers lying between the Delta of the Orinoco and the Pomeroon River, *A. aquasalis* is the vector. This zoophilic and exophilic mosquito bites man and enters houses frequently; this is probably related to the absence of livestock. DDT reduced the incidence of malaria drastically, but sporadic cases continued to occur. In the latter part of 1958 a prolonged and severe drought caused river waters to turn brackish — by tidal infiltration — over a much greater portion of their lower courses. This produced an unusual surge of *A. aquasalis;* a sharp increase in the incidence of Malaria followed. Cases also occurred on the Venezuelan side of the border, but in all probability these originated from British Guiana as the Orinoco Delta and adjoining Venezuelan territory have been declared to be eradicated areas for some years past.

4) The upper reaches of the rivers in the North West District, where *A. darlingi* was the vector, remain in general free from malaria. Recently however, CHARLES has identified a focus of endemic malaria at Koriabo on the upper Barima River, which he attributes to transmission by *A. bellator,* an exophilic, bromelia breeder.

The residual malaria problems which persist in British Guiana thus involve a population of only 20,000 living in remote parts of the interior out of a total of 518,000 inhabitants. Failure of DDT spraying in these remote areas is related either to exophilic habits of carriers, other than *A. darlingi,* or to habitual frequent displacements of the Amerindian population to situations where residual methods are inapplicable and where *A. darlingi* exists as a semi-wild mosquito attacking animals but still maintaining its preference

for human blood when available. Lack of control across the Brazilian border is obviously a factor in the Rupununi-Takatu Savannahs.

Surveillance in these remote, sparsely inhabited areas, with little or no facilities for communication and transportation, is extremely difficult, costly and practically impossible during the rainy season when transmission is usually at its peak. Control of the human reservoir of infection by the systematic, periodical distribution of antimalarial drugs is equally impractical. It is being practiced at present under great difficulties in the North West area along the Venezuelan Frontier and on the Pomeroon river.

The isolation of these remote areas of the interior, and the extremely restricted channels through which communication and shipment of supplies must flow, seem on the contrary to offer favourable conditions for the use of Pinotti's Medicated Salt technique (Pinotti 1954; Pinotti et al. 1954). Control of the salt supply should in fact be easy; no natural deposits exist in the interior and no substitutes are used by the Indians who use and appreciate this commodity both for seasoning and preserving fish and meat. Through the normal trade channels, pyrimethaminised or chloroquinosed salt should reach even the remotest Amerindian and miners camps lost in the wilderness.

A plan for the systematic medication of all salt supplies proceeding to the North West District, to the Upper Cuyuni, Mazaruni and Potaro Rivers and to the Rupununi District has been prepared in cooperation with the Pan American Health Organisation and W.H.O. It is hoped that it will be put into practice in the very near future.

In the course of malaria eradication campaigns, particularly in areas of heavy endemicity, progress tends to be rapid and often spectacular during the earlier phases; as the incidence of the disease is curbed, however, new and often unsuspected epidemiological problems tend to emerge and require solution. It has been said that it is the reduction of the last 10% in the incidence of malaria that consitutes the ultimate proving test of any eradication campaign. When that stage is reached, in backward countries where rural medical facilities are absent or rudimentary, the difficulties of evaluation become enormous, for a variety of reasons:

(1) Infected persons are few and far between and their detection becomes more and more difficult and relatively more costly.

(2) Trained and experienced professional personnel become less available; experienced specialists tend to move on to more fertile fields; their substitutes, with less and sometimes no experience, have little or no opportunity to improve their knowledge owing to the overwhelming proportion of negative field observations and laboratory findings; soon other public health activities of a more actual and pressing nature absorb their energies and interests. The residual malaria problems which constitute the difference between control and eradication tend to be overlooked or neglected.

(3) Technicians tend to be left to their own devices in the highly frustrating routine of examining thousands of negative slides. Training of technicians becomes impossible and when sent away to be trained the overwhelmingly negative routine rapidly kills their interest and alertness.

(4) It does not take long for clinical experience of malaria to be lost. In British Guiana today, the majority of medical practitioners have had no personal experience of the disease.

(5) With the reduction of malaria incidence to minimal proportions, the problem of total eradication loses much of its appeal to administrators, politicians and the public in general. The sums required for adequate surveillance appear disproportionate in respect to the small number of cases diagnosed and therefore expenditure tends to be regarded as wasteful in the presence of other obvious and apparently more pressing needs. Trained surveillance personnel is partly or totally diverted to other functions.

The medicated salt technique introduced by PINOTTI has given encouraging results in the field trials carried out in Brazil; the effectiveness of the method has been corroborated under strict experimental conditions in volunteers by G. R. COATNEY and his co-workers (COATNEY et al 1958). The highly « automatic » character of the technique should overcome many of the difficulties which harass the late stages of malaria eradication campaign, *provided that commercial salt is generally and habitually used by the inhabitants.* Under the difficult conditions existing in the remoter inhabited areas of the Guiana interior, a trial application of Pinotti's method over 3 or 4 years offers the best promise towards the achievement of ultimate eradication of the small foci of malaria still surviving in the remoter parts of the territory.

QUINDICI ANNI DI ESPERIENZA NELL'ERADICAZIONE DELLA MALARIA NELLA GUIANA INGLESE. MEZZI DI MANTENIMENTO E PROBLEMI RESIDUI

La campagna antimalarica nella Guiana Inglese, iniziata nel 1945, ebbe come risultato l'eradicazione dalla zona costiera, densamente abitata (450.000 ab.), non solo della malaria, già iperendemica, ma anche del suo vettore *A. darlingi*. Questi risultati erano già manifesti nel 1948 e sono stati mantenuti con pieno successo negli ultimi quindici anni; le operazioni di irrorazione sulla costa sono state sospese fin dal 1951. Ciò fu reso possibile, nonostante il carattere continentale della regione, a causa delle specifiche esigenze di *A. darlingi* quanto a luoghi di riproduzione, ed all'esistenza tra la regione costiera alluvionale densamente abitata e l'immensa foresta che copre l'interno di due peculiari formazioni geologiche che producono acque superficiali troppo acide per lo sviluppo di *A. darlingi*. Queste barriere naturali sono state integrate per mezzo di periodiche irrorazioni con DDT delle case, strettamente limitate ai banchi alluvionali dei corsi d'acqua che attraversano queste formazioni.

Nell'interno (circa 60.000 ab.) la malaria è stata pure eradicata dalle aree più accessibili e densamente popolate, sulle sponde dei fiumi raggiunte dalla marea (circa 30.000 ab.). Solo nei più remoti distretti dell'interno esistono ancora i pro-

blemi della malaria residua, interessanti una popolazione di soli 20.000 abitanti largamente disseminati. Questi insuccessi delle tecniche dei trattamenti ad affetto residuo sono in relazione alle condizioni locali di trasmissione dovute a vettori esofili diversi da *A. darlingi* (*A. aquasalis* e *A. bellator*); ai frequenti spostamenti della popolazione amerindia che passa una parte considerevole della sua esistenza in ricoveri temporanei, non trattabili, nella foresta; alla mancanza di misure di controllo nel territorio brasiliano dall'altra parte della frontiera.

Le estreme difficoltà che si hanno per raggiungere questa piccola popolazione largamente disseminata in un immenso ed impervio territorio rendono impossibile la sorveglianza di routine e la distribuzione sistematica di medicamenti antimalarici; è stato preparato un piano, con la collaborazione dell'Organizzazione Sanitaria Pan-Americana e dell'O.M.S., per l'eradicazione definitiva della malaria da queste aree remote a mezzo dell'incorporazione di medicamenti antimalarici nelle provviste di sale da cucina, secondo la tecnica introdotta da Pinotti.

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POST ERADICATION MALARIOLOGY

CLAY G. HUFF (*)

A plea is made to maintain basic research on malaria as if eradication were only a very distant goal. It is believed that such an attitude would (1) help to assure the continued training of malariologists and (2) increase the chances that the broad field of intracellular parasitism would advance without interruption.

In this number of the *Rivista di Parassitologia,* devoted to tributes to the memory of the late Professor GIUSEPPE BASTIANELLI, it seems appropriate to give some consideration to the future of malariology as a science. Because of the devastations which the malarial parasites have brought to mankind, malariology has never needed an excuse for its existence. Malariologists could always count as one of their chief motivations the desire to contribute to the reduction of the ravages of this disease upon man. This applied (and still applies) to all persons who worked on any phase of the subject — clinicians, pathologists, entomologists, parasitologists, pharmacologists, and chemists. With the success of malarial eradication in many countries of the world and with eradication of malaria from the entire world a possibility, we now must realize that one motivation for studying malariology is diminishing and may eventually disappear. What future does malariology have in the light of these possibilities?

The immediate future should, of course, be concerned with a greatly intensified effort on the part of all interested persons to speed the removal of human malaria from the list of man's greatest enemies. Since the nature of that effort differs in no essential from that which is being actively pursued today we shall not concern ourselves here with it, but shall give some thought to what may be the need for continuing some aspects of malariology as a science after the goal for eradication of human malaria may have been accomplished.

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It should be fairly apparent from the preceding statements that malariology is considered here as embracing more than the study of human malaria. Otherwise, the eradication of human malaria would not only remove the motivation for studying malariology but also its subjects. Malariology is considered here as the study of malarial parasites. With the eradication of those malarial parasites which affect man there will remain a very large number of species which infect animals — some very closely related to the human malarial parasites, many others less closely related to them but, nevertheless, closely enough related to them to have been very important in the history of the development of our knowledge of malaria.

Unless the eventual eradication of human malaria should be delayed beyond what now seems a reasonable period for its accomplishment, many of the basic problems will be unsolved when the last malarial parasite of man has disappeared from the earth. Will these basic problems be important enough to justify the continuation of their study through the use of the malarial parasites of animals? It is my contention that they are, and this opinion is based upon the belief that science progresses most satisfactorily through the pursuit of research on the broadest possible base. Many of the principles still obscure in the problems of malariology will need to be clarified before we can understand the basic relationships in cellular biology. This is not to say that the study of malarial parasites will miraculously supply the *whole* answer to these basic relationships. The majority of the answers can and will be supplied through research done on other types of organisms. However, we shall never be able to understand parasites like the malarial parasites without studying parasitism.

The most rapid strides in biological discovery have been made by the wise choice of organisms for experimental studies. In genetics, *Drosophila;* in cancer research, the mouse; in virology, the chick embryo; in immunology, the guinea pig, are examples of this practice. However, after these rapid strides have been made we have usually found that we have only an incomplete view of a field. The use of other organisms in similar studies have yielded results different in important aspects from the original, quickly obtained results using the carefully chosen animal. The genetics of *Paramecium* is only a part of protozoan genetics; the physiology of *Tetrahymena* is not the whole story for all protozoa; the population studies, the studies on sex, the cytology, the behavior of one kind of organism does not represent the whole of knowledge for all members of the same systematic group to which the experimental subject belongs. These statements are trite and undisputed. I make them only because there seems to be a blind spot in the attitude toward some of the information which we need about obligate parasites — and particularly intracellular parasites. We shall not know the nature of the sexual process in *Plasmodium* until it has been worked out in species of this genus. We shall not know the com-

plete physiology of *Plasmodium* through the study of free-living organisms. Many of the unsolved problems concerning the immunological response to malarial parasites, the factors which bring about the production of sexual cells from asexual cells, the principles governing the selection of host cell types by malarial parasites and many others will not be solved by working upon other organisms. I venture to predict that in the much desired era in which human malaria will have been banished from our midst we shall still have good reasons for wanting to know the answers to some of the problems of parasitism which can best be answered through research on malarial parasites.

Obligate parasites are more difficult to study than free-living organisms because of the difficulty of growing them *in vitro* and of their inaccessibility *in vivo*. This fact undoubtedly explains their comparative neglect and the consequent lack of knowledge of the principles of parasitism. There is, however, no easy path to better understanding of them. Largely because of their great medical importance more effort has been given to research on malarial parasites than to other intracellular protozoa. It would be unwise to allow the eradication of human malaria to interrupt the steady progress which has been made in improving our methods of the study and in advancing our knowledge of a group of organisms so well suited to the study of intracellular parasitism as are the malarial parasites.

Basic research upon malarial parasites should continue for two reasons. First, eradication of the disease has not been accomplished and it is conceivable and even probable that unforseen obstacles will arise to delay its consummation. Second, continuation of the study of intracellular parasitism through the use of malarial parasites as subjects would be more likely to advance more rapidly than by turning to parasitic organisms much less well studied. We should not underestimate the importance which attaches to the present skill in handling malarial parasites in the laboratory. Among the animal malarias we have a wide choice of species exhibiting a large variety of characteristics. Their vertebrate hosts include lizards, birds, rodents, bats, and primates. Many of their insect vectors are now easily bred in the laboratory, thus making it possible to propagate the parasites sexually as well as asexually. Growth of several of the parasites is now successfully carried out in chick embryos — either as erythrocytic or as exoerythrocytic infections. In recent years tissue culture has been greatly improved as a method for growing the exoerythrocytic stages of some of the species parasitic in birds and this has made possible the study of the living parasite through phase microscopy and time-lapse cinephotomicrography. Real advances have been made in the study of the physiology of the erythrocytic stages and the way has been largely cleared for similar studies of the exoerythrocytic stages. Immunological and chemotherapeutic techniques are available for manipulating and directing

the course of experiments in a variety of ways. Progress has been made in the growth of exogenous stages *in vitro*. Growth has been obtained of exoerythrocytic stages enclosed in diffusion chambers placed in nonsusceptible as well as susceptible hosts. Growth of exogenous stages in the hemocoele of mosquitoes now opens the way to the experimental study of this portion of the cycle. This does not exhaust the list of newer methods and knowledge now available to the experimentalist but it does indicate the opportune situation obtaining in regard to the possibility for an exciting and fruitful growth of malariology as a basic discipline.

It is to be hoped that funds will continue to be made available for the devolopment of our knowledge in this field regardless of the question of the eradication of human malaria. Categorical research is yielding to research directed to the understanding of principles. For this reason it should be emphasized that the disappearance of malariology *per se* would be of no consequence — once the human disease has been eradicated. On the other hand, the continued use of malarial parasites as tools for advancing our knowledge on a broad front is desirable if not essential.

LA MALARIOLOGIA DOPO L'ERADICAZIONE

Con la possibilità all'orizzonte della eradicazione della malaria umana, è essenziale che qualche idea sia dedicata alla preservazione e continuazione delle ricerche basilari in questo campo per il tempo in cui la malaria umana avrà cessato di fornire la principale ragione per tali ricerche. Nella persuasione che il progresso scientifico si realizza in modo migliore attraverso il simultaneo progredire di quante più delle sue branche è possibile, si auspica la continuazione dello studio dei parassiti malarici degli animali a quello stesso alto livello a cui potrebbe essere mantenuto ove la possibilità dell'eradicazione della malaria fosse affatto remota. Le ricerche di base sui parassiti malarici dovrebbero continuare; in primo luogo, per assicurare la continuazione dell'addestramento di nuovo personale nella conoscenza teorica e nelle tecniche della malaria fino a che l'eradicazione non sia stata realizzata, ed ,in secondo luogo, per utilizzare le capacità presentemente acquisite nel trattare questi parassiti ai fini del progresso del vasto argomento del parassitismo intracellulare.

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BASTIANELLI, EIN WEGBEREITER DER MODERNEN MALARIATHERAPIE

WALTER KIKUTH (*)

Der Artikel würdigt BASTIANELLIS Verdienste auf dem Gebiete der Malariologie. Mit Nachdruck werden BASTIANELLIS Bemühungen hinsichtlich der klinischen und epidemiologischen Entwicklung der ersten synthetischen Malariaheilmittel hervorgehoben.

Ebenso wie Sir PATRICK MANSON schon zu Beginn seiner ärztlichen Laufbahn allen Fragen der Malariatherapie die größte Aufmerksamkeit schenkte und dieser Neigung bis zu seinem Lebensende treu blieb, befasste sich auch BASTIANELLI, der große italienische Meister der Malariologie, dem wir auf allen Gebieten der Malaria so unendlich viel zu verdanken haben, während seines ganzen Lebens mit großer Intensität theoretisch und praktisch mit den Problemen der Verhütung und Behandlung dieser Krankheit. Er war einer der ersten, der vorurteilslos die neuen synthetischen Malariaheilmittel einer kritisch-wissenschaftlichen Betrachtung unterzog und auf Grund eigener umfassender Erfahrungen den Wert der modernen Therapie frühzeitig erkannte, die eine neue Aera der Malariatherapie einleitete, welche während des zweiten Weltkrieges ihre größten Triumphe feierte.

Es sei nicht vergessen, dass BASTIANELLI in den unter der Aegide des Völkerbundes stehenden Fortbildungskursen, welche in dem von ihm geleiteten Institut für Malariologie « E. Marchiafava » stattfanden, die Erfinder der neuen Malariamittel zu Wort kommen ließ, um im wechselseitigen Gespräch die Ergebnisse des Laboratoriums mit denen der Praxis in Einklang zu bringen. Die Anregungen, die hier seinerzeit von ihm und seinen Mitarbeitern RAFFAELE, MOSNA und CANALIS ausgingen, haben nicht nur für die weitere Entwicklung der Chemotherapie reiche Früchte getragen, sondern, wie es sich später herausstellte, auch unsere Ansichten über den Lebenszyklus

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der Malaria reformiert und inzwischen nach zwanzigjähriger Forscherarbeit durch die Entdeckung der exoerythrozytären Formen der Malariaplasmodien einen erfolgreichen Abschluß gefunden. Auch an dieser fortschreitenden Entdeckung hat RAFFAELE, ein Schüler BASTIANELLIS, einen ganz wesentlichen Anteil gehabt, denn es gelang ihm, bei der Vogelmalaria (*P. elongatum* u. *P. relictum*) ausserhalb der roten Blutkörperchen gelegene Stadien der Malariaparasiten zu finden. Er war ganz eindeutig der erste, der eine gedankliche Verbindung zwischen den Sporozoiten und den neu entdeckten Entwicklungsstadien der Plasmodien herstellte.

Der Chemotherapeut, der sich die Aufgabe stellt, neue Malariamittel zu entwickeln, ist in einer unselbständigen Lage. Er ist nicht nur auf die Mitarbeit des Chemikers angewiesen, der ihm die synthetischen Mittel zur Verfügung stellt, sondern in gleichem Maße auf die Mitarbeit des Klinikers, der allein den Wert eines Medikamentes für die Praxis beurteilen kann. Es braucht wohl hier nicht näher begründet zu werden, warum ein neues synthetisches Mittel ohne vorausgehende Tierversuche nicht unmittelbar auf seinen therapeutischen Wert geprüft werden kann. Bei der Auswahl geeigneter Testversuche stößt der Chemotherapeut immer wieder auf Schwierigkeiten, die oft nicht leicht zu überwinden sind. Das trifft für die Malaria im besonderen Maße zu, denn die Plasmodien der menschlichen Malaria lassen sich auf die für experimentelle Arbeiten in Frage kommenden Tiere nicht übertragen.

Wie so oft in der Wissenschaft, hat auch auf dem Gebiete der Malaria eine vorausgehende vorurteilslose Grundlagenforschung den Bestrebungen einer Zweckforschung unschätzbare Dienste geleistet. 1911 fasste ein junger griechischer Arzt, COPANARIS, der damals auf der von GIEMSA geleiteten chemischen Abteilung des Hamburger Tropeninstitutes seine wissenschaftlichen Kenntnisse erweiterte, den Gedanken, die beim Menschen gegen die Malaria wirksamen Substanzen, Chinin und Salvarsan, bei mit *P. relictum* infizierten Kanarienvögeln auf ihren chemotherapeutischen Wert zu prüfen. Er konnte die von ihm vermutete Wirksamkeit beim Chinin bestätigen, während das Salvarsan sich als unwirksam erwies. Dieser grundlegenden Feststellung von COPANARIS wurde anfangs nur geringe Bedeutung beigemessen; es ahnte damals niemand, dass seine Deduktionen ein völlig neues Gebiet erschlossen hatten, das zum Ausgangspunkt einer weltweiten Forschung werden sollte. Fussend auf diesem Testmodell der Vogelmalaria prüfte ROEHL, ein Schüler PAUL EHRLICHS, in systematischer planvoller Arbeit gewissermassen in Symbiose mit den Chemikern serienweise eine große Anzahl synthetischer Verbindungen, welche peroral mit einer Schlundsonde den infizierten Vögeln verabreicht wurden. Bei der Synthese dieser neuen Verbindungen war das Methylenblau, das bereits von MARKS bei der Vogelmalaria als therapeutisch wirksam erkannt worden war, richtunggebend. ROEHL fand in dem von SCHULEMANN,

Schönhöfer und Wingler synthetisch hergestellten Plasmochin ein dem Chinin therapeutisch überlegenes Mittel.

Die klinische Prüfung des Plasmochins brachte Ueberraschungen, die aus der Sicht des Laboratoriums nicht vorauszusehen waren. Zwar erwies es sich als therapeutisch wirksam, aber doch in einer ganz anderen Art und Weise als das Chinin. Während Chinin auf die Schizonten aller drei Malariaarten einwirkt, war das Plasmochin dem Chinin in dieser Beziehung unterlegen, zeigte aber ganz unerwartet eine dem Chinin fehlende Fähigkeit, die Gameten der Malaria tropica zu vernichten. Eine korrespondierende Wirkung der Empfänglichkeit zwischen den Erregern der Vogelmalaria und den menschlichen Plasmodien war beim Plasmochin nicht vorhanden.

In Weiterverfolgung des nun einmal eingeschlagenen Weges, auf der Suche nach neuen und besseren Mitteln, wurde von mir der Roehlsche Testversuch modifiziert und durch Hinzuziehung der Haemoproteus-Infektion der Reisfinken ergänzt. Mit Hilfe der kombinierten Testmethode der Vogelmalaria einerseits und der Haemoproteus-Infektion andererseits gelang es mir, bei einer von Mietzsch und Mauss hergestellten Acridinverbindung, die später den Namen Atebrin erhielt, den Nachweis einer chininähnlichen Wirkung aufzuzeigen, obwohl diese Verbindung bei der Vogelmalaria dem Plasmochin weit unterlegen war. Bei der klinischen Prüfung erwies sich Atebrin in der Tat als ein Malariamittel, das in gleicher Weise wie Chinin bei allen drei Plasmodienarten auf die Schizonten einwirkt, auf welche alle klinischen Symptome zurückgeführt werden können.

Von nun an konnten Chinin und Atebrin als Schizontenmittel bezeichnet werden, Plasmochin als ein Medikament mit gametoziden Eigenschaften.

Um den therapeutischen Wert des Atebrin in vollem Umfang zu erkennen, bedurfte es einer langjährigen klinischen Prüfung. Eine solche exakte Durchführung therapeutischer Versuche stößt in der Praxis vielfach auf unvorhergesehene Schwierigkeiten. Ein neues Mittel muß bei allen Malariaarten auf seine Verträglichkeit und Wirksamkeit in therapeutischer und prophylaktischer Beziehung bei zahlreichen Erwachsenen, Kindern und Säuglingen unter den verschiedensten klimatischen und epidemiologischen Bedingungen und unter voneinander abweichenden Milieufaktoren geprüft werden. Auch die unterschiedliche Empfänglichkeit der verschiedenen Stämme ein und derselben Plasmodienart darf nicht ausser acht gelassen werden, denn eine endemische Malaria tertiana auf Sizilien kann sich klinisch und therapeutisch anders verhalten als ein Stamm derselben Art in Südafrika.

Diese jahrelange Prüfung der Malariaheilmittel wurde auf breiter Basis in den verschiedensten Ländern der Welt in Gang gesetzt. Unter den vielen Forschern, die sich dieser Aufgabe widmeten, ist es wiederum Bastianelli, dem wir in besonders hohem Maße Dank schulden. Im Jahre 1935 übernahm Bastianelli die Leitung einer Forschergruppe, der seine Schüler Mosna und

Canalis angehörten, welche im Auftrage der Malariakommission des Völkerbundes in groβen Feldversuchen in den Dörfern Posada und Torpé auf Sardinien die synthetischen Malariaheilmittel Plasmochin und Atebrin allein und in Kombination miteinander bei einer groβen Zahl von Malariakranken aller Altersstufen auf ihren prophylaktischen und therapeutischen Wert analysierte.

Die Ergebnisse dieses planvoll angelegten Feldversuches sind von Bastianelli kritisch bewertet worden, wobei er mit Nachdruck hervorhob, dass die Vernichtung der Plasmodien auf dem direkten Wege erfolgt und eine Beeinflussung der Immunitätslage der Patienten durch die Behandlung nicht zu befürchten ist. In einer zusammenfassenden Arbeit sind diese Ergebnisse von Mosna und Canalis im Fourth General Report der Malariakommission des Völkerbundes 1937 unter dem Titel "Prevention and Treatment of Malaria by Synthetic Drugs" veröffentlicht worden. Der günstigen Beurteilung von dieser Seite gesellten sich die Stimmen der Prüfer aus anderen Ländern, so dass zu Beginn des zweiten Weltkrieges ein festes Fundament von Erfahrungen über die Anwendung der synthetischen Mittel bei der Malaria vorlag.

Im Kriege hat sich das Atebrin als ein hervorragendes Mittel zur Beherrschung dieser Seuche erwiesen. Unzählige Menschen auf den verschiedensten Kontinenten verdanken Atebrin Leben und Gesundheit. Die Kriegserfahrungen haben gezeigt, dass mit Hilfe des Atebrin Menschen ohne Malaria-Immunität in der Lage sind, auch in hyperendemischen Malariagebieten zu leben und groβen Strapazen ausgesetzt werden können, ohne an Malaria zu erkranken.

Während die Vorzüge des Atebrin allgemeine Anerkennung gefunden hatten, konnten die Nachteile der neuen Therapie nicht übersehen werden. Bei der Tertiana muβte nach wie vor mit einer grösseren Anzahl von Rückfällen gerechnet werden; das Atebrin war offensichtlich als Vorbeugungsmittel kausal-prophylaktisch unwirksam, was bei der Vogelmalaria bereits von mir und Giovannola festgestelt worden war. Unangenehm wurde ferner die eintretende Gelbfärbung der Haut und der Skleren bei den behandelten Patienten empfunden, und gelegentlich wurden auch unangenehme Nebenerscheinungen beobachtet. So wurde die Suche nach Mitteln mit besserer Wiksamkeit und Verträglichkeit fortgesetzt.

Bei diesen Bestrebungen wurde mit der klassischen, von mir modifizierten und erweiterten Testmethode Roehls, von Andersag und mir und unabhängig von uns von den Amerikanern eine halogen substituierte Chinolinverbindung, das Resochin (Cloroquine), als wirksamstes Präparat entdeckt, dem die Nachteile des Atebrin nicht mehr anhafteten und das heute als eines der besten Malariaheilmittel ganz allgemein Anerkennung gefunden hat. Den englischen Chemikern Curd und Rose glückte die Synthese eines Präparates von ganz neuem Typ, eines Biguanids, das den Namen Paludrin erhielt, welches neben seiner schizontoziden Wirkung auch einen echten gegen die

exoerythrozytären Entwicklungsstadien gerichteten kausal - prophylaktischen Effekt erkennen läßt, allerdings nur bei den sich verhältnismässig schnell entwickelnden E. Stadien von *P. falciparum.*

Als Plasmochinersatz wurde später ein Präparat gleichfalls aus der Plasmochinreihe mit besonders guter Verträglichkeit, das Primaquine, in die Therapie eingeführt. Der Wirkung des Resochins entspricht auch das Camoquine, ein Chinolinpräparat mit einer basischen Seitenkette in der 4-Stellung des Chinolinringes.

Mit den synthetischen Mitteln ist es heute möglich, die Malaria in allen ihren Erscheinungsformen mit einer vor Beginn der exakten chemotherapeutischen Forschung für unmöglich erachteten Schnelligkeit und Sicherheit zu heilen. Diesen unerwarteten und sensationellen Erfolg der experimentellen Chemotherapie, den BASTIANELLI noch erlebt hat, verdanken wir zahlreichen Forschern aus vielen Ländern der Welt und nicht zuletzt ihm und seinen Schülern, die wesentlich zum Erfolge beigetragen haben.

Damit wären wohl fast alle Wünsche, die wir Aerzte und Hygieniker in Bezug auf die chemotherapeutische Bekämpfung der Malaria hatten, in Erfüllung gegangen. Und doch wird man auf dieser Plattform des Erreichten nicht stehenbleiben können, sondern nach weiteren Verbesserungen der Therapie und in besonderem Maße nach einer zuverlässigen und leicht durchzuführenden Prophylaxe streben. Solche Bemühungen werden an vielen Stellen intesiv fortgesetzt. Die WHO ist nicht nur bestrebt, die Malaria zu bekämpfen, sondern das Ziel dieser Organisation ist die Ausrottung dieser Seuche. Mit den kontaktinsektiziden Mitteln im Rahmen der Mückenbekämpfung ist man auf diesem Wege schon sehr weit gekommen, aber man ist sich auch darüber im klaren, dass man mit dieser Methode allein das Ziel nicht erreichen kann. In den letzten Jahren hat man sogar bei einzelnen Anophelesarten im Zuge der DDT-Bekämpfung eine Resistenz gagen die modernen Kontaktinsektizide festgestellt. In derartigen und ähnlich gelagerten Fällen könnte die Chemoprophylaxe die Mückenbekämpfung vikariierend unterstützen. Eine solche Bekämpfung wäre aussichtsreich, wenn es gelänge, ein prophylaktisches Mittel zu finden, welches bei einmaliger Verabreichung imstande wäre, gefährdete Personen für einen längeren Zeitraum vor der Sporozoiten-Invasion zu schützen und gleichzeitig eine schon bestehende Infektion zu kupieren. Auch auf diesem Sektor wäre eine Zusammenarbeit zwischen Chemotherapeuten und Malariologen von unschätzbarem Wert. Hoffen wir, dass dieses weitgesteckte Ziel in naher Zukunft erreicht wird.

BASTIANELLI, PROMOTORE DELLA MODERNA CHEMIOTERAPIA ANTIMALARICA

Questo articolo rende omaggio ai meriti di BASTIANELLI nel campo della malariologia. Viene posta in evidenza la parte avuta da BASTIANELLI nella valutazione clinica ed epidemiologica dei primi preparati antimalarici sintetici.

DR. BASTIANELLI, A PROMOTER OF MODERN ANTI-MALARIAL CHEMOTHERAPY

The article pays homage to BASTIANELLIS merits in the field of Malariology. Emphasis is put on BASTIANELLIS roll with respect to the clinical and epidemiological evaluation of the first synthetic anti-malarial drugs.

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MALARIA IN ANCIENT GREECE

G. A. Livadas (*)

Dealing with the subject of malaria in ancient Greece, the writer remarks that the view of the existence of this disease in Greece since prehistoric times is not well grounded. In the writer's opinion, the first clear and indisputable information of this fact is to be found in the Orphics, which were written in the 6th century B.C., and in Hippocrates' period (466-377 B.C.), malaria was widespread and well known in the country.

The time at which malaria appeared in Greece cannot be precisely determined. On the basis of various traditions and legends, preserved in ancient Greek texts, it has been claimed that this disease was ravaging the country from time immemorial. So, according to Kardamatis (1), the slaying of Lernean Hydra by Hercules symbolizes the efforts made in prehistoric times to sanitate the Argos plain from malaria, which mainly consisted in draining the standing water of Lerna swamp to the sea (**). The same writer states (1) that the myth about the killing of the Stymphalian birds by Hercules symbolizes an analogous sanitation effort that was attempted by excavating the underground sinkholes which were feeding River Erasinus. On the other hand, many of the epidemics, described by ancient Greek writers as having occurred in very old times, may, according to Kouzis (4), have been due to malaria, such as, for instance, the Keos outbreak of 1250 B.C., the Aegina epidemic prior to the capture of Troy, the Thebes one at Oedipus'period and the Beotian outbreak before the building of Heraclea.

Of the epidemics reported by ancient Greek writers, Kouzis (4), considers that the one very corresponding to a malaria outbreak is that described by Diodorus of Sicily which is said to have afflicted the areas opposite to the Islands of Rhodes and Chios at the time Leucippe arrived in Rhodes.

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(**) It would not perhaps be out of place to remind here that the small lake which still bears the same name as above constituted as late as a few years ago a serious source of malaria in the surrounding area (2).

According to this description, (*) the epidemic was preceded by a deluge which caused great calamities and, finally, owing to the pollution of the air (mal-aria), an epidemic broke out in the cities. According to KARDAMATIS (1), the ruins found in Beotia of very ancient drainage works, which are attributed to the people of Minyae that fluorished before the Trojan War, are evidence of an attempt not only to improve the land for agricultural purposes but also to sanitate the highly unhealthy area of Lake Copais, where the above people had settled from Thessaly.

In SRABO's works (**), reference is also made to the effarts used in this area for the above purpose (***).

SAVVAS (5) in his pertinent historical review, relying on the above mentioned sources, accepts the view that malaria was prevalent in Greece even in that very ancient period. Finally, according to KOUZIS (4) and KARDAMATIS (3), the reference made in two passages of Iliad to epidemics corresponds to malaria outbreaks. and, in the above writers' opinion, this constitutes a further indication that malaria must have been known in Greece at the time the Homeric epic poems were written.

In the first (****) of these passages mention is made of an epidemic that broke out in the Achaean camp and caused heavy disaster.

The other passage (*****) is more important and refers to disastrous epidemics which usually occur in autumn and cause high fevers to mortals.

(*) ''τὴν δ'ἀντίπεραν τῶν νήσων κατ'ἐκείνους τούς καιρούς συνέβη διά τόν κατακλυσμόν μεγάλας καί δεινάς κάτασχεῖν ἀτυχίας · διά μέν γὰρ τὰς ἐπομβρίας ἐπὶ πολλοὺς χρόνους ἐφθαρμένων τῶν καρπῶν σπάνις τε τῶν ἐπιτηδείων ὑπῆρχε καί λοιμικὴ κατάστασις ἐπεῖχε τὰς πόλεις δια τὴν τοῦ ἀέρος φθοράν,,

Diodorus of Sicily V, 82, 1. Edition C. Müllerus.

(**) ''τὸ χωρίον ὅπερ ἡ λίμνη κατέχει νῦν Κωπαὶς ἀναψύχθαι πρότερον καὶ γεωργεῖσθαι παντοδαπῶς ὑπὸ τοῖς 'Ορχομενίοις οἳ πλησίον οἰκοῦσι καὶ τουτ 'ἦν τεκμήριον τοῦ πλούτου τιθέκσι,,

Strabo IV, 2, 40.

(***) Attempts to drain Lake Copais were repeatedly made over the centuries — from the period of Alexander the Great to our own era. The endemicity of the area however remained high, as Ronald Ross had a chance of ascertaining on his visit there; and it was only recently, following the great malaria campaign of the period 1946-1949 that the above area got completely rid of malaria for the first time in its history.

(****) ''νοῦσον ἀνὰ στρατὸν ὦρσε κακὴν, ὀλέκοντο δὲ λαοί''.

Iliad, A, 10.

(*****) ''τὸν δ'ὸ γέρων Πρίαμος πρῶτος ἴδεν ὀφθαλμοῖσιν
παμφαίνονθ' ὥστ'ἀστέρα ἔπεσσύμενον πεδίοιο
ὅς ῥα τ'ὀπώρης εἰσίν ἀρίζηλοι δὲ αἳ αὐγαὶ
φαίνονται πολλοῖσι μετ'ἀστράσι νυκτός ἀμολγῷ
ὅν τε κυν' 'Ωρίωνος ἐπίκλησιν καλέουσιν'
λαμπρότατος μέν ὅδ'ἐστι ἰκακὸν δέ τε σῆμα τετυκται
καί τε φέρει πολλὸν πυρετὸν δειλοῖσι βροτοῖσιν,,.

Iliad, X.

This latter case was deemed as possibly corresponding to the autumnal malaria outbreaks which, as known, were usual in mediterranean countries.

* * *

In any case, it is evident that the above stated views on the appearance of malaria in ancient Greece, being based on dubious and inadequate data, can only be taken as mere hypotheses.

The vagueness of the sources from which the relevant information emanates, the distortion caused to the actual events under the influence of popular imagination and the intervening time, the complete ignorance of etiology of the various morbid conditions that prevailed in the very ancient period to which the said information refers, and the complete inability that existed at that time, for lack of knowledge and facilities, to differentiate the various morbid conditions — all this explains to some extent why it is difficult to draw, under these circumstances, positive conclusions in this matter.

The first clear information on the presence of malaria in Greece is to be found in the Orphics which are thought to have been written by mystic religiouns poets.

In these books a full description is given of malaria symptoms, and a clear distinction is also made between the various forms of the disease, tertian and quartan. And, what is still more remarkable, the writers of Orphics knew well certain particular characteristics of these forms. Specifically, they speak of the long duration of the quartan form and its resistance to the therapeutic means used (*). Accordingly, if the suported view (6) that the Orphics were written in the 6th century B.C. is correct, it is reasonable to believe that malaria was well know in Greece at that period. Later, it seems that the disease spread to the Greek cites in Sicily — a fact that may be presumed from the descriptions of the epidemics that broke out in those cities during the 5th century B.C., and, particularly, from the reported succes of the sanitary works carried out to control these epidemics.

Empedocles (504 B.C.) was, in all probability, the first to conceive, in the historic times, the idea of the unfavourable effect of standing water on the health of the neighbouring dwellers, and who devised the execution of various drainage works to eliminate the ill consequence thereof.

He, according to Plutarch (**), succeeded in ridding his native town,

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(*) "εἰ δέ πυριφλεγέθων ἑτερήμερος ἄνδρα θαμίζων ἢ κρυερός μάρπτων πυρετός, παρενήνοθε γυίοις ἠὲ τεταρταίης πῆμα βραδὺ μήποτε λήγειν βουλομένης ἀλλ'αἰὲν ὅπη πελάσησι μενούσης τὸν δὲ οὐγ'ἰήσασθαι δι'ἀμύμονος αὐτίκ'ἀχάτου οὗτος γάρ πυρετῶν πολύ φέρτερος,,.
Orphics. Litica, 627.

(**) "'Εμπεδοκλῆς τὴν τε χώραν ἀπηλλάξεν ἀκαρπίας καί λοιμοῦ διασφᾶγας ὄρους ἀποτειχίσας, δι'ὧν ὁ νότος εἰς τό πεδίον ὑπερὲβαλλε,,.
Plutarch to Kolotis, II, Edition Xylandrus.

Agrigente, of a pestilence outbreak, and was able to do so by fencing a mountain chasm, through which the waters of the sea used to flood the plain when south winds were blowing.

As reported by Diogenes of Laerte (*), Empedocles saved also the town of Selinonte from a disastrous pestilence by diverting the flew of two adjacent rivers and eliminating the swamps surrounding that town.

In the period of Hippocrates (466-337 B.C.), malaria was already widespread in Greece. This is clearly gathered from the detailed descriptions of this disease contained in the Hippocratic collection.

Hippocrates first described, in a masterly way, the different feverish types of malaria, distinguishing them into continuous, quotidian, tertian and quartan (**).

He also described the malignant form of the disease and pointed out the severity these assume in childhood, usually appearing with convulsions (***).

From his descriptions of the country of Phasienae, Hippocrates seems to have also been aware not only of the complications observed in chronic conditions, such as splenomegaly but also of the aftereffects of long residence near the swamps (cachexy, hydropsy) (****).

Moreover, Hippocrates noted also the effect of morfological factors on the occurrence of periodic malaria outbreaks (*****), as well as the increase in incidence observed after a rainy summer (******).

Finally, Hippocrates knew also well blackwater fever, and described a considerable number of cases of this syndrome that sometimes follows malaria (*******).

From what has been stated above, it is made clear that malaria in the

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(*) "τοῖς δέ Σελινουντίοις ἐμπεσόντος λοιμοῦ διά τάς ἀπό τοῦ παρακειμένου ποταμοῦ δυσωδίας ὥστε καί αὐτούς φθείρεσθαι καί τάς γυνάϊκας δυστοκεῖν, ἐπινοῆσαι τόν Ἐμπεδοκλέα καί δύο τινάς ποταμούς τῶν σύνεγγυς ἐπαγαγεῖν ἰδίαις δαπάναις καί καταμίξαντα γλυκαῖναι τά ῥεύματα' οὕτω δέ λήξαντος τοῦ λοιμοῦ,,.
Diogenes of Laerte. H. H. 20.

(**) Hippocrates. Edition Kühn III.

(***) Hippocrates. Edition Kühn II.

(****) Ὡς ἐκ τῆς ἐν τοῖς ἕλεσι διαίτης "οἱ κάτοικοι τὴν τε χροιὰν ὠχρὴν ἔχουσι ὥσπερ ὑπὸ ἰκτέρου ἐχόμενοι,,.
"ἔνιοι τῶν σπληνιώντων, ὑπὸ μὲν τῶν φαρμάκων πίνοντες οὐκ ὠφελέονται οὐδ'ὑπό ἄλλης θεραπείας οὐδ'ἰσχνότερος γίνεται αὐτῶν ὁ σπλήν,,. "προιόντος δὲ τοῦ χρόνου ἐνίοισι μὲν εἰς ὕδρωπα περιΐσταται ἡ νόσος καὶ διεφθάρησαν,,.
Hippocrates. Edition Kühn, II.

(*****) Hippocrates. Edition Pournaropoulos, III, Aphorisms.

(******) "ἦν δὲ τὸ θέρος ἔπομβρον γίνεται καὶ νότιον τὸ μετόπωρον χειμῶνα ἀνάγκη νοσερὸν εἶναι,,
Hippocrates. Edition Kühn, I.

(*******) Hippocrates. Edition Kühn, III.

classical times was well known and prevalent in Greece. Later there was an impetuous spreading and increase of incidence in the country.

To this there may have contributed the hardships which followed the Peloponnesian war and the destruction of forest, started at that time and carried on thereafter (*), which, according to existing evidence (**) were until that period preserved in excellent condition, to such an extent as to provide abode for bears (***) and lions (****).

It is indeed by no means improbable that to this widespread prevalence of malaria is largely due, as believed by various writers (Jones (7), Ross (8) (9) and Sanarelli (10)), the subsequent decline and final collapse of the Ancient Greek civilization.

(*) Pausanias. V, 4, 6.
(**) Herodotus. V, 82.
(***) Pausanias. Attica.

(****) "πορευομένῳ δὲ ταύτῃ (τοῦ Περσικοῦ στρατεύματος) λέοντες οἱ ἐπεθήκοντο τοῖσι σιτοφόροισι κἀμήλιοι... ,,.
Herodotus. VII, 126.

LA MALARIA NELLA GRECIA ANTICA

In relazione al problema della malaria nella Grecia antica, l'A. espone anzitutto il punto di vista sostenuto dai più antichi scrittori che la malaria era già diffusa in Grecia in tempi preistorici. Secondo la sua opinione queste vedute, basate su dati incerti ed insufficienti, quali quelli forniti da varie leggende e tradizioni conservate in antichi testi greci, sono pure ipotesi, e ciò rende quindi impossibile la precisa determinazione dell'epoca in cui la malaria è comparsa in Grecia.

La prima chiara ed incontestabile notizia sulla malaria in Grecia si trova, secondo l'A., negli Orfici che si ritengono essere stati scritti da scrittori mistici nel VI sec. a C.. Al tempo di Ippocrate (466-377 a. C.) la malattia era già ben conosciuta e largamente diffusa in Grecia. Quest'ultimo fatto, come precisato dall'A., è dedotto dalle dettagliate descrizioni delle varie forme cliniche della malaria che si trovano nelle opere di Ippocrate, e dalle importanti informazioni date nelle stesse circa l'epidemiologia della malattia. Nel testo sono anche riportate citazioni dei principali passaggi delle più importanti opere greche antiche.

L'A. afferma infine che l'aumento dell'incidenza della malaria e la sua diffusione generale dopo la guerra peloponnesiaca hanno probabilmente contribuito, come ritenuto da precedenti scrittori, al declino ed alla caduta dell'antica civiltà greca.

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THE DYNAMICS OF RESISTANCE TO INSECTICIDES BY ANOPHELINES

GEORGE MACDONALD (*)

Selection of a recessive characteristic such as resistance to DDT is necessarily slow and could be greatly delayed by reduction of selection pressure, but selection of a dominant characteristic such as dieldrin resistance is rapid, and could only be materially delayed by virtual elimination of pressure. The qualities of mixtures of DDT and dieldrin for reduction of this selection are explored and it is concluded that there might be special advantages in their use both for the control of normal insects and the prevention of DDT resistance, but not for the prevention of dieldrin resistance.

Two forms of physiological resistance to insecticides by anopheline mosquitoes have been thoroughly studied in the Ross Institute laboratories and reported on by DAVIDSON (1958). They concern resistance to DDT by *Anopheles sundaicus* and resistance to dieldrin, benzene hexachloride and certain other insecticides by *A. gambiae*. There are a number of other strains and species under examination in the laboratory, the findings on which so far have not in any way upset the preliminary conclusions on these two fully studied examples, which may perhaps be taken as a reasonable representation of many happenings throughout the world. The two forms of resistance studied are quite distinct from each other, a finding which indicates the probability that the two insecticides act in different manners from each other. In both cases inheritance is by a single gene mechanism, the significance of this now well established fact lying in its indication of the method of selection by favoured breeding from pre-existing heterozygote or homozygote individuals, in which the characteristic must have been as well marked as it is in present day laboratory maintained strains. The original appearance of these genes must have been as mutants, but there is no indication of any great frequency of this mutation,

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and there are reasons to think that mutation to dieldrin resistance, which is now known to be widespread in *A. gambiae* in West Africa but is certainly rare in other parts of Africa, may be only very occasional. It may well be that some other attribute which is associated with this gene may be detrimental to the prospects of the insect's survival in many environments but less so in some parts of West Africa, where the occurrence of a mutation which is only very occasional has resulted in the persistence of the characteristic in the anopheline population.

Resistance to DDT in *A. sundaicus* is recessive, the heterozygote closely resembles the susceptible homozygote and so closely that it may reasonably be inferred that it succumbs to normal field doses of insecticide in about the same proportions as does the homozygote susceptible. The resistant homozygote however, probably survives field applications of insecticide almost unscathed. The selection of resistance to DDT is therefore probably the result of the preferential survival of homozygotes alone, the heterozygotes suffering the same fate as the normal susceptible insects.

On the other hand, resistance to dieldrin and BHC is partly dominant, the heterozygote being intermediate between the two homozygote forms. So far as normal field practice is concerned, however, it is probable that the relatively strong resistance of the heterozygote results in its escaping destruction by insecticides almost as completely as the homozygote resistant form. In considering selection in the field therefore, resistance to dieldrin and BHC may be regarded as fully dominant.

The processes of selection of a recessive characteristic such as DDT resistance, and a dominant characteristic such as dieldrin resistance, are radically different in degree though they are the same in broad general principle. The original distribution of homozygotes and heterozygotes in both cases may be assumed to follow the Hardy Weinberg law, according to which the homozygote is prevalent in proportion to the square of the frequency of the gene in the population, and becomes extremely rare when the gene frequency is low. The process of selection in favour of a recessive characteristic, starting from a low frequency of the gene, involves the destruction of the vast majority of its carriers in the heterozygote form and the selective survival of only some very small proportion as homozygotes. Selection in favour of a dominant gene, again from an original low gene frequency, involves the selective survival from the start of all carriers of the gene, with the result that the process of multiplication is rapid from the first and immeasurably more speedy than in the contrasting example of selection of a recessive.

The selection of individuals resistant to dieldrin and BHC may conveniently be considered first. The ratio of the number of offspring produced by the dominant resistant form to those produced by the recessive susceptibles, $1 : (1 - s)$, describes the relative fitness or survival value of the two types

of individual, and s is called the selection co-efficient. It might be representative of common field conditions that the resistant individual should have ten times the survival value of the susceptible in which case the value of the selection co-efficient would be 0.9.

If p and q are the proportions of the dominant and recessive genes respectively to the whole gene pool, so that $p + q = 1$ and that p^2, $2pq$, and q^2 represent the proportions of the dominant homozygote, the heterozygote, and the recessive homozygote, then the amount of change in the gene constitution of the population in the course of one generation is given by the well established expression $\triangle q = q_1 - q_2 = \frac{-sq^2(1-q)}{1-sq^2}$

There is no precise general solution to the sequence of which this is the derivative and the detailed working of population dynamics demands much laborious serial calculation. However, when the value of q is either very small or very large certain simplifications are permissible and they are applicable to the study of the early phases of selection of resistance. When q is very large and is to be reduced by the selection of the contrasting dominant gene, the above expression may be simplified to $\triangle q \backsimeq \frac{s(1-q)}{1s} = \frac{s-p}{1-s}$ and the number of generations lapsing between any two successive values of p. the amount of the dominant gene, is represented by the expression $n \backsimeq \frac{\log p_2 - \log p_1}{\log s - \log (1-s)}$. These abbreviations are applicable to the case of selection of resistance to dieldrin and BHC, a dominant characteristic initially rare in the population, and may be used for values of p below 0.001, after which resort must be made to serial calculations. Under the type of selection pressure which has been suggested the frequency of the dominant resistant gene would increase nearly ten times in each generation, perhaps from little more than one millionth part of the total gene pool to one thousandth in three generations. After this the rate of multiplication would be slightly reduced but still extremely rapid; values of p, the frequency of the dominant gene, in the next six successive generations being 0.0098, 0.084, 0.34, 0.56, 0.69 and 0.75. This represents the emergence of the characteristic from extreme rarity to predominance in less than ten generations and conforms with the field happenings that this type of resistance has made itself apparent very soon after the application of insecticides. Reduction of the intensity of selection pressure would of course result in a slower rate of emergence but it would remain very speedy at any of the degrees of pressure which can be visualised in general insecticidal practice.

When the value of q is very small the expression for the rate of change in the frequency of the gene can be simplified to $\triangle q \backsimeq -sq^2$ and when this applies the number of generations intervening between two successive values of q are given by $n \backsimeq \frac{1}{s}\left(\frac{1}{q_2} - \frac{1}{q_1}\right)$ Resistance to DDT is a recessive

characteristic and the frequency of the gene conveying it may be considered as q in this case. The simplified expressions being permissible for values of q below 0.01 or 1 per cent. In this case the carrier of the recessive gene has a greater chance of survival than the dominant and the selection co-efficient, s, therefore has a negative value. A comparable degree of selection to that considered in relation to dieldrin resistance would be indicated by a selection co-efficient with a value of —9 which gives the ratio $1 : (1 - s)$ a value of 10. Selection of this type which involves the destruction of a great proportion of gene carriers as heterozygotes is very slow. At the levels suggested about 1,000 generations would elapse before the value of q increased from 0.0001 to 0.001 and another 100 generations before it increased to 0.01. Another 5 generations would see it increased to 0.02, a further 2 generations to 0.03, after which the values in successive generations would be 0.04, 0.05, 0.075, 0.12, 0.22, 0.81, 0.97 and 0.997. An extremely slow original multiplication, which might be spread over very many years if the gene were originally rare, leads in the end to a rapid increase which would appear to be dramatically sudden in its onset, because a recessive gene would be unlikely to be observed before it had a moderately high frequency but soon after would become dominant in the population. This is in accord with the known happenings in places where resistance to DDT has appeared, several years of uninterrupted progress having been suddenly brought to an end by the dramatic appearance of resistance.

It may also be noted that dieldrin resistance emerges with extreme rapidity from even the most minute origins, and it is reasonable to infer where several years' application of this insecticide has not resulted in emergence of the resistance that the gene is probably absent from the anophelines of the district. However, in the case of DDT resistance the fact that it has emerged after relatively so short a time as four years, which are not likely to include more than on hundred generations, show that the gene must have been present from the first in some considerable frequency though concealed in its recessive heterozygous form. Slightly lower original frequencies or somewhat reduced rates of selection pressure would greatly prolong this original period of apparent freedom from the characteristic, and it is a reasonable working assumption that the gene is very widespread in the anopheline population but that in most places the degree and time of selection pressure have not been sufficient to bring it forward. Further cases of the emergence of this characteristic are therefore to be expected even in places which have considered themselves free for many years.

Prevention

The only method of preventing the emergence of these forms of resistance is by reduction of the degree of selection pressure. Consideration of the cases

given above shows that the selection pressure in favour of dieldrin-BHC resistance would have to be virtually eliminated if the time for emergence of the characteristic were to be prolonged sufficiently to make its appearance unimportant. On the other hand, reduction in the degree of selection pressure in favour of DDT resistance might be expected to result in great prolongation of the period of emergence of the characteristic.

The only feasible way of securing this reduction other than abandonment of the use of the insecticide concerned is by the use of mixtures of insecticides arranged to ensure that some notable mortality is inflicted on resistant individuals. Exploration of the qualities of such mixtures is therefore an urgent need in the present situation, and must go through successive stages of preliminary laboratory trial, amplified testing under conditions resembling those found in the field, and field trials. Solution of the problem depends on both the technical and the economic aspects of the use of mixtures, and it is only possible to study the latter under field conditions which are beyond the scope of ordinary laboratory practise.

The experiments which are here recorded are intended as a contribution to the laboratory studies of this subject.

Experiments with Mixtures of Insecticides

Materials and methods.

The work was carried out in the Ross Institute insectaries at a standard temperature of 26°C. and a relative humidity of 80° using anopheline mosquitoes reared under standard conditions and routinely tested when two days old. Testing was carried out by the Busvine-Nash technique using 1-hour exposure followed by a period of 24-hours before observation of results. Those here included refer only to the findings of female mosquitoes after 24 hours, but the findings on males and immediately after exposure were recorded and show no serious discrepancy from those here reported. Controls were put up for all tests and the results have been standardised for mortality amongst them, which was small. The strains of mosquitoes used were the Lagos strain of *Anopheles gambiae* which is established to be a pure strain normally susceptible to insecticides, the B. K. strain of *A. gambiae* which consists of homozygotes resistant to dieldrin, BHC and some other related insecticides and an Indonesian strain of *A. sundaicus* which is a homozygote resistant to DDT. The experiments were made in parallel with others in which the degree of this resistance was established (see Davidson *loc. cit.*) and it was therefore not considered necessary to re-establish it in the course of the present enquiry.

Mixtures of dieldrin and gamma BHC were used, at first in a variety of proportions at normally spaced concentrations. Early experience led to a

simplification by concentrating on one ratio between them, a DDT: dieldrin ratio of 10 : 1, and to a modification in the method of spacing the concentrations used. It had soon become clear that a very narrow spacing was needed between the concentrations to be used to avoid abrupt moves from negligible to almost total kills in two consecutive tubes, and so to allow reasonable interpretation of the results. A logarithmic series was adopted in which each succeeding dose exceeded its predecessor by approximately 26 %. This is a convenient series, actually with the logarithm of each concentration exceeding that of

TABLE 1.

Records of results of exposure of female anophelines to serial doses of DDT dieldrin, and mixed DDT and dieldrin, by Busvine Nash technique. Control mortalities for individual tests were all low, and in sum less than 2 per cent.

Insecticide		*A. gambiae*			*A. gambiae* B. K. Resistant to DLD			*A. sundaicus* Indonesia Resistant to DDT		
DDT %	DLD %	No. exposed	No. dead	Mortality %	No. exposed	No. dead	Mortality %	No. exposed	No. dead	Mortality %
0.4		34	6	18						
0.5		105	45	43	12	0				
0.63		47	23	49	13	2				
0.79		53	30	57	24	11	46			
1.0		90	66	73	27	9	33			
1.25		68	67	98	170	116	68			
1.6		65	64	98	118	116	98			
2.00		24	24	100	98	95	97			
	0.032	36	4	11				9	1	11
	0.04	37	21	57						
	0.05	63	40	73				15	6	40
	0.063	38	23	74				24	17	79
	0.079	57	46	81				154	86	56
	0.10	29	27	93				171	141	83
	0.125	73	73	100				83	83	100
	0.160							93	91	98
	0.20	32	32	100						
0.32	0.032							16	1	6
0.4	0.04	28	11	39						
0.5	0.05	57	34	60	15	1	11	24	16	67
0.63	0.063	47	43	100	20	6	30	29	22	76
0.79	0.079	549	549	91	28	16	56	36	32	89
1.0	0.10	23	23	100	41	26	63	228	222	97
1.25	0.125				171	128	75	108	108	100
1.60	0.16				110	107	97			
2.0	0.20	35	35	100	39	39	100			

the previous one by 0.1. It has the advantage of being endlessly repetitive, running in terms of percentage, 0.01, 0.0125, 0.016, 0.02, 0.025, 0.032, 0.040, 0.050, 0.063, 0.079, 0.1, 0.125, etc. etc.. Separation on a logarithmic or multiplication scale such as this is rational; the intervals between concentrations are small; there are ten concentrations in a ten-fold increase whether that be from 0.01 % to 0.1 % or from 0.05 % to 0.5 %, and the repetitive form makes it simple to memorise the scale and take it up at any point. This scale was used in making up solutions of single materials and proved easily workable in practice, facilitating the making of very fine distinctions in the effects of different doses. When using mixtures of insecticides each was calculated on this scale, a mixture containing, for instance, 0.05 % dieldrin and 0.5 % DDT or 0.1 % dieldrin and 1 % DDT.

TABLE 2.

Regression coefficients for female anophelines tested against single and mixed insecticides by the Busvine-Nash technique.

Anopheline	Insecticide	Probit value of expected mortality, Y	LC_{50}
A. gambiae, Lagos (susceptible)	DDT	5.7 + 4.3 log. dose	0.68 %
	dieldrin	10.6 + 4.1 log. dose	0.043%
	mixed DDT & dieldrin	8.2 + 10.4 log. dose	0.45 % DDT & 0.045% dieldrin
A. gambiae B. K. (resistant to dieldrin)	DDT	4.9 + 7.7. log. dose	1.0 %
	mixed DDT & dieldrin	5.0 + 9.7 log. dose DDT	1.0 % DDT
A. sundaicus (resistant to DDT)	dieldrin	10.8 × 4.9 log. dose	0.07 %
	mixed DDT & dieldrin	12.1 + 5.8 log. dose dieldrin	0.06 % dieldrin

These expressions are given as logarithms of the actual dose, which in this series is usually a negative quantity because most of the concentrations are less than 1%. To reconvert to the more usual log. (dose × 10) subtract b from a in the expression $Y = a + b$ log. dose.

Results

The results in so far as they refer to female mosquitoes are set out in Table I and the expressions of the regression co-efficients are set out in Table II. The findings for the normally susceptible Lagos strain of *A. gambiae* closely resemble previous ones, giving a picture which is now becoming accepted as generally representative for most anophelines. Dieldrin is about 16 times more potent a toxicant to this strain than is DDT and the variance of susceptibility around the mean is about the same for the two materials. This variance is shown by the value of b in the regression equation $Y = a + b$ log dose, and in the text which follows it is interpreted to give the percentage of insects which one would expect to survive a dose equal to 1½ times the LC_{50}. With the variance shown for *A. gambiae* about 23 % would survice 1.5 × LC_{50} and about 10% would survive 2.0 × LC_{50}.

The findings resulting from exposure of the normally susceptible Lagos strain of *A. gambiae* to a mixture of the two insecticides gives results which differ both in degree and in principle from those resulting from the application of one alone. The LC_{50} of 0.045 % dieldrin and 0.45 % DDT is at first sight disappointingly high, being almost exactly what would have been expected from the dieldrin content alone. The consistency of these findings of the relatively ineffective nature of small doses throughout various tests makes them very probably real and independent of serious laboratory error. However, in contrast to this, no single mosquito of this strain has yet been observed to survive exposure in the tube containing 0.79% DDT and 0.079% dieldrin, which is only 1.7 times as great as the LC_{50}, and in addition to the 549 female mosquitoes observed, 490 males submitted to this dose have all also died. Even the narrow spacing of the concentrations which was adopted has proved too broad for proper measurement of this phenomenon, because only 4 of 47 mosquitoes exposed to 0.63 % DDT and 0.063 % dieldrin survived, and it would have been interesting to have observed results at even more narrowly spaced concentrations.

The B. K. strain of *A. gambiae,* which is homozygous resistant to dieldrin and BHC, is somewhat less susceptible to DDT than the Lagos strain but the variance around the mean susceptibility is low and only about 9 % would be expected to survive 1.5 × LC_{50}. The chi square test shows that the probability of the distinction from the Lagos strain being due to random error only is less than 0.00001.

The reaction of this same strain to the mixture of DDT and dieldrin is much the same as would be expected from its DDT content alone except that again the variance is reduced and under 5 % would be expected to survive exposure to 1.5 × LC_{50}. Again this difference has been established as real by the chi square test. *A. sundaicus,* homozygote resistant to DDT, is very similar

to the Lagos strain of *A. gambiae* in its reaction to dieldrin to which it is susceptible and has only a slightly higher LC_{50} with a similar variance. About 20 % would survive exposure to 1.5 × LC_{50}. It is slightly more sensitive to a mixture than would have been expected from its dieldrin content alone. Although this difference is not very great, it is real, and the probability of its being due to chance is less than 0.0001. The variance is slightly less than it is in relation to dieldrin alone and about 16 % would be expected to survive 1.5 × LC_{50}.

Discussion

The results of combining insecticides have been considered at length by Plackett and Hewlett (1948), and by Hewlett and Plackett (1950), who considerably elaborated previous explorations and described four varieties of relationship between the components and the mortality to be expected from their use. These depended on whether the lethal action of the two poisons was exerted at the same point in the physiological mechanism and whether the metabolic characteristics which make an individual insect relatively hardy in relation to one poison have the same or an opposing, or an unrelated effect in relation to the other. The general form of the usual distribution of mortality from insecticides around a mean is well understood and is represented by a straight regression line connecting the probit mortality and the logarithm of the dose, expressing an ever increasing risk of mortality with increasing dose without however implying the concept of an LC_{100}. The findings which are here presented on the use of a single insecticide, or of a mixture of insecticides against an insect known to be resistant to one of them, conform to this general pattern. The findings following exposure of normally susceptible *A. gambiae* to a mixture may however well represent a totally different pattern corresponding to Hewlett & Plackett's description of independent joint action. This is the state when toxicants elicit a common response, but do so when administered jointly by causing totally separate and distinct physiological mechanisms to fail, and which do not mutually interfere with their actions by the one altering the action of the other or the reaction of the insect to it. In this state the mortality which is to be expected from the use of a mixture of the two insecticides represents the sum of the mortalities which might be expected from the use of each of the insecticides alone, or 100 %, whichever is the lower. This state is represented by a markedly curved probit mortality/log dose regression line and by the occurrence of a definite LC_{100}, concentrations at or above which kill all insects exposed without either practical or theoretical probability of survival. Conformity of the present results with this condition is not quite complete and the best approximation is that $m = 0.68\ (m_1 + m_2)$ where m, m_1 and m_2

represent the proportionate mortality expected from the mixture and from the two insecticides used independently.

The authors refused to discuss the utility of mixtures of insecticides and conclude that pairs which have this characteristic of independence of action are much to be desired, partly because they can be much more certainly effective insecticides and partly because their use might tend to prevent the development of resistant strains. These arguments cannot be applied directly to the present case because they regarded resistance as being of a polyfactorial nature, as was then generally considered to be the case.

It seems clear that if a mixture of insecticides were to be used it would, if properly applied, prove to be an extremely effective method of controlling susceptible mosquitoes, possibly much more effective than any of those now in use and with reasonably probability of killing a very high proportion of the mosquitoes exposed to it. If there were in this population any mosquitoes homozygote resistant to DDT they would experience a notably lower but still very considerable mortality. Examination of the regression line indicates the probability that the concentration of the mixture of insecticides which would kill 80 % of susceptible insects would also kill about 36 % of any homozygote DDT resistant members of the population as compared with a mortality of perhaps 6 % amongst the latter if DDT alone had been used. The expectations of life conforming to these habitual mortality rates would be in the ratios 1.95 : 3.3 : 16.15 or 1 : 1.7 : 8.3. It is not legitimate to take numerical statement much further than this because it is not reasonable to equate the probability of reproduction with expectation of life when low values of the latter are considered, particularly in anophelines in which reproduction does not occur until after survival for five days. It is therefore reasonable to say that the use of a mixture might be expected to produce a really notable reduction in the selection pressure and thus a notable increase in the time before the demonstrable emergence of resistance, but laboratory studies cannot take the analysis of the time factor much further than this.

The prospects of prevention of emergence of resistance to dieldrin and BHC are much more remote. Examination of the regression line suggests that the mortality inflicted on any homozygote or heterozygote resistant individuals might be very much less than that inflicted on the susceptibles and might indeed remain relatively insignificant. The dose of mixture causing the death of 80% of normally susceptible insects might increase the death rate amongst dieldrin resistant individuals by no more than 5 % or 10 %, and therefore produce only produce a slight amelioration in selection pressure. When this fact is taken in conjunction with the great speed of normal selection of a dominant characteristic such as this and the need for the virtual total elimination of selection pressure to delay it materially, it appears

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SOME PATHOLOGICAL PROCESSES IN MALARIA AS EXEMPLIFIED IN THE DEVELOPMENT OF HEPATIC LESIONS IN ACUTE INFECTIONS

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The pathological processes initiated by the parasitic infection of the erythrocyte lead to hepatic dysfunction and sometimes to centrilobular necrosis. The mechanisms underlying these changes are both specific to the parasite and non-specific. The former apparently depend on the dissemination of some soluble substance. The latter are often common to many acute medical states and include some form of interference with oxygen usage by tissue cells and dynamic vascular reactions which in the liver lead to constriction of the hepatic venous tree and the consequent production of centrilobular stagnant anoxia. There is some evidence that these circulatory phenomena may be reflex in origin; they may occur with or without concomitant generalized circulatory failure The vascular patterns of the liver are discussed in this connection.

The pathogenesis of malaria is discussed in this article with special reference to the liver lesions, but it should be appreciated that although liver damage is specifically mentioned the processes discussed are often basically similar in other organs and in the host as a whole.

The function of the liver is disturbed in all forms of malaria (1, 2, 3). In acute and complicated malaria, the disturbance of function is maximal and may be accompanied by structural damage which presents in the form of cellular degeneration and necrosis in the central zone of the lobule. The hepatic lesion derives from a combination of specific and non-specific processes of which the latter are probably the most important. The pathological processes are set in motion by the fact of parasitization, but we do not yet know the connection between the parasite in the erythrocyte and the tissues of the disturbed host. We are aware, however, of some of the factors which contribute towards the development of the pathological patterns of the

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disease. Each of these processes may be active in itself, but a combination of them can apparently produce effects which each process singly could not accomplish. For example, as will be seen below, cellular metabolic damage may have serious local consequences but these are considerably enhanced by concomitant processes, such as interference with the local circulation of blood, to evolve an additive effect which neither factor alone could produce.

Although in the final analysis the factor initiating the changes in tissue must be the parasitization of the erythrocyte, many of the pathological processes thus initiated are largely determined locally by the anatomical and physiological structure of the tissues concerned and are not specific to malaria but are common to many other forms of acute disease. There are certain other features which are of course specific to malaria, for instance the production of malarial pigment, the effects of the destruction of erythrocytes at schizogony and the metabolic competition which may develop between the parasite and host. In addition, there are secondary processes deriving from the developing disturbances in function and structure and which in turn may exert general or local effects. The disease is thus evolved from a complex of non-specific and specific primary and secondary processes, all of which are set in motion directly or indirectly by the parasitization of the host erythrocyte. The initiating factors are unknown, but the evidence, as we shall see, points strongly to some chemical agency.

In the development of these processes the element of time is of great significance. Their progress is controlled not only by the intrinsic intensity of the factors involved but also by the time over which they act. In the case of the liver, for instance, the advancement of tissue damage to the point of structural change is dependent on the stress to which the tissue is exposed multiplied by the time over which it acts. This is true of many forms of liver damage besides that produced by malaria and has been demonstrated experimentally in the anoxaemic perfused liver (4, 5).

Clinical and biochemical evidence of functional damage to the liver has been advanced by many authors (1, 2, 3). There is general agreement that early damage to the liver commonly presents in the form of deviations of various « liver function » tests, indicating disturbances in certain metabolic processes in which the liver is involved. There is no point in cataloguing such evidence here. It is well-known, for instance, that bilirubin may accumulate in the plasma. In the early stages of acute malaria the production of albumin may be retarded as a result of hepatic functional disturbance and at a later stage other proteins are disturbed. There are also indications that the detoxifying powers of the liver parenchyma are reduced, a point which may be of considerable importance in so far as tissue demage is concerned, since active substances normally detoxified may, under such circumstances, escape destruction.

Functional hepatic disturbance may or may not be accompanied by structural change in the liver tissue. In very severe infections there is usually clear evidence of tissue damage; in milder infections there may be none.

The earliest changes in the hepatic cells are detectable in tissue stained with haematoxylin and eosin, in which the normally bluish-pink cytoplasm of the epithelium becomes more eosinophilic and granular. Fatty degeneration may follow in the cells on the periphery of the lobule and necrosis in the centrally placed cells.

Changes in lobular structure are usually manifest only late in acute infections. The picture in the early stages is one of dilatation of the sinusoids and central veins with some slowly developing central changes depending on the severity of the infection, not only in terms of the number of parasites but also in regard to the pathological reactions of the host. There may be little obvious histological alteration of the parenchyma at this stage. The liver cells are normal or slightly subnormal in size and not swollen. The Kupffer are swollen and show signs of active phagocytosis of erythrocites, parasitized and unparasitized, pigment debris, etc; they do not mechanically obstruct the sinusoids. The advanced lesion, which may come on very rapidly especially in haemolytic infections, is one of centrilobular necrosis with dilatation of the central vein and sinusoids. The cellular necrosis occurs first about the central vein and spreads towards the periphery; the peripheral cells often show signs of fatty degeneration but do not become necrotic.

This centrilobular lesion is not specific to malaria or to blackwater fever, but is common to many other conditions of acute medical nature. The lesion itself is thus basically non-specific to malaria.

Nevertheless, the plasmodial infection must institute the processes leading to it. We are at present ignorant of the early initiating stages but have some knowledge of the way in which the lesion develops.

The production of the centrilobular changes can best be explained by some kind of obstruction to the flow of blood in that area. Complete obstruction of flow to the whole lobule could be induced in numerous ways but in this case the circulation to the lobule appears to be disturbed in such a way that the central zone becomes the most affected.

The picture suggests that in some way the blood flow at the periphery the lobule is maintained whereas that through the centre is impeded. In other words, the central drainage of the lobule is disturbed. If this were merely a matter of obstructed lobular drainage, the whole lobular circulation might be expected to become involved. The fact that it does not indicates alternative circulatory pathways at the lobular periphery (6. 26).

The part played by local circulatory factors in the production of centrilobular lesions has recently been reviewed by ANDREWS (6) who concluded that the evidence strongly supports the view that some differential circulatory

disturbance is involved, in which the flow in the central region of the lobule is impeded whereas that in the peripheral region is partly maintained. The mechanisms leading to this circulatory readjustment are still under discussion.

MAEGRAITH and his colleagues contend that an active dynamic process is involved whereby the drainage from the lobule is impeded by active constriction of the hepatic venous tree, the lobular circulation being maintained at the periphery through vascular connections described by ANDREWS and others (1, 2, 7, 8, 9).

Other authors have suggested that the central flow is more likely to be impeded passively by mechanical factors, including swelling of the hepatic cells and « blocking » of the sinusoidal flow.

The evidence favours the dynamic hypothesis. Active constrinction of the hepatic veins has been demonstrated in the intact animals and in the perfused livers of many animals. It has also been demonstrated in man (6, 8).

Recent research by RAY and his colleagues has given further support to the view that hepatic venous constriction is involved in the production of the centrilobular lesion and has suggested that it may be of nervous origin; evidence of reflex hepatic venous constriction has also been reported by ANDREWS (10, 11, 12, 13). Thus, RAY has reported that centrilobular necrosis does not develop in knowlesi infection in sympathectomized rhesus monkeys, whereas it is usual in infected normal monkeys. He has recently demonstrated that the circulatory disturbances may be dependent on a reflex pathway which involves the postero-lateral region of the hypothalamus.

The same author has presented evidence that the fatty changes seen in the peripheral lobular cells are produced by processes independent of those which cause the centrilobular damage. He found that fatty changes could be induced in the periportal cells in rhesus monkeys by the intravenous injection of plasma from animals infected with *P. knowlesi* and could be minimized by the contemporaneous administration of choline. Injection of the plasma did not, however, lead to central necrosis and choline had no effect on the latter. The deduction was that the plasma of infected animals contained some soluble substance capable of producing fatty degeneration in the peripherally-placed cells but not necrosis.

RAY and his colleagues observed that transfusion of whole blood or of erythrocytes could impede the production of centrilobular changes in infected monkeys, and suggested that the lesions might be in some way related to a prevailing anoxaemia setting up, either locally or through the hypothalamus, a chain of reactions which ultimately operated hepatic venous constriction and the consequent changes in the lobular blood flow. Further work is required in this field, but there seems little doubt that there is some nervous control of the hepatic vessels, including the hepatic veins and that

constriction of these vessel can be initiated by various conditions, including the development of medical or surgical shock (1, 7, 19).

Possible alternative mechanisms which could limit the circulation to the centre of the lobule may be important in certain conditions in which the same ultimate pathological pattern is produced, but are not adequate to explain these lesions in malaria. For instance, the impedence of the flow to the centre in carbon tetrachloride poisoning appears to be due largely to obstruction of the sinusoidal flow by swollen polygonal cells, which first show swelling and hydropic changes in the mid-zone and not in the central zone of the lobule, thus mechanically limiting the blood flow to the central region of the lobule, in which the cells rapidly become necrotic (14, 15). In malaria this is not the case. Occasional swelling of polygonal cells has been reported in liver biopsy material from *P. vivax* infections, but we have never seen it in biopsy or autopsy of knowlesi malaria or in other acute malaria infections.

It is possible that some mechanical obstruction may occasionally occur in advanced infections from « packing » of the sinusoids with swollen Kupffer cells, free phagocytes, « sludge » and so on, but there is little evidence that this is a significant factor in the development of the malarial centrilobular lesion as such (1, 16), nor is there any evidence to support the suggestion that the sinusoids themselves may limit the flow by constricting independently (17).

Injury to the hepatic venous tree (for example, endophlebitis and thrombosis as seen in Chiari's disease or in veno-occlusive disease) may also produce centrilobular changes of a very similar nature, but such changes do not occur in malaria, in which thrombosis or stasis of the hepatic vessels is exceedingly rare.

It would seem therefore that the failure of the central blood flow in the hepatic lobule in acute malaria is difficult to explain on a purely mechanical basis. The dynamic hypothesis is more satisfactory and more easily fits in with the wide distribution of the centrilobular lesion in other acute medical states. There has been some criticism of the concept of active hepatic venous constriction based on the view that the hepatic veins in many animals do not have the power to constrict and that their constriction, even in dogs, is dependent on « sphincters » of smooth muscle developed at the outlets of the hepatic veins into the vena cava. Recent studies on perfusion on the canine liver and on the behaviour of the vessels in the intact animal have demonstrated however that even the smaller branches of the hepatic veins can constrict readily in the dog and in other animals, including man. Moreover, smooth muscle may be demonstrated in these minute venous branches in many animals by suitable histological techniques (6).

The neurological mechanisms possibly involved in such constriction in the intact animal have already been mentioned. The results of experiments

on the perfused liver indicate that constriction of the hepatic venous tree may also be brought about without the intervention of the central nervous system, in a manner not unlike the similar effects in the renal vessels.

The initiation of these dynamic vascular effects in malaria is not yet understood, nor is the effect on the liver tissues of centrilobular ischaemia or stagnant anoxia *per se*. In some animals the cellular processes may be much more advanced than in others, even with roughly the same degree of parasitic infection. It would seem likely, therefore, that other factors may be involved, including, as pointed out below, metabolic changes in the hepatic cells themselves, possibly involving all the cells and not specifically those in the central zones. In such a case, the summation of the cellular changes plus those of the local circulatory stagnation may lead to extensive damage in the centrally placed cells which might otherwise have escaped or have become involved to the same extent only as the cells of the rest of the lobule.

At any one time the circulatory changes may be the major factor, at another the cellular changes. In veno-occlusive disease for instance, the pathological obstruction to the venous flow is clearly the dominant factor in developing the lesion. In carbon tetrachloride poisoning the central sinusoidal circulatory failure in also a vital factor, although initiated in this instance by swelling of the mid-zonal liver cells. In malaria it would seem that both factors are important in that the prevailing cellular dysfunction is often by itself ineffective in producing necrosis but may do so when the central circulation becomes impeded.

It is important to appreciate that the cellular dysfunction in malaria is usually detectable long before any obvious deviations in hepatic circulation have occurred. There are probably many reasons for this early cellular disturbance. One factor which could conceivably affect the hepatic cells in malaria, in which the erythrocytes themselves are involved, might be disturbance of the physico-chemical properties of haemoglobin in so far as its powers of oxygen acceptance and discharge are concerned. Anaemia itself may also effect some changes in the activity of the liver cells, but in acute malaria a degree of anaemia is seldom reached in which the carriage of oxygen falls short of that required for basic metabolic processes. In association with local circulatory disturbances, however, anaemia may become a significant factor. Over the last ten years we have studied the behaviour of haemoglobin in most forms of mammalian malaria and have found in every instance that the properties of the pigment are unaffected at any stage of the disease (19). So long as the pulmonary circulation is adequate, oxygen uptake and transference by haemoglobin continues and the discharge at the tissue face is unaffected since, except in the ultimate terminal stages, the composition of the plasma does not alter sufficiently to deviate the oxyhaemoglobin dissoci-

ation curve, except occasionally to the right (which facilitates rather than retards the dissociation) (1, 19).

If, in the absence of circulatory disturbances, the oxygenation of the blood and the discharge of oxygen at the tissue face are adequate, as would appear from our studies on malaria, it is probable that the genesis of necrosis may lie in the failure of the cells to accept or to use oxygen. We have investigated this point in *P. berghei* infection in mice (20). Suspensions of mitochondria isolated from liver cells of infected animals have shown, when compared with those of normal animals, a decreased oxygen usage with an associated fall in P : O ratio. This effect was observed with two substrates involving DPN in the oxidative pathway and one wich did not. Addition of DPN stimulated oxygen consumption in the former, and resulted in a partial stimulation of associated phosphorylation. The biochemical lesion which was thus demonstrated was primarily concerned with the oxidative phosphorylating functions of the mitochondria and was most developed about the fifteenth day, at a time when there was severe anaemia. Similar but less pronounced effects have been recorded in otherwise normal mice after prolonged severe haemorrhagic anaemia, which must be presumed to be a major factor in their production.

It appears, therefore, that in berghei malaria changes in enzymatic functions occur in the hepatic cell not unlike those reported after carbon tetrachloride poisoning and arising from somewhat similar mitochondrial damage. It is not clear whether this is partly a specific effect due to the malaria or entirely a secondary one arising from other factors set in motion by the infection. An examination of the problem in *P. knowlesi* infections is now in progress.

This study, of course, covers only one side of the problem. Functional disturbance is not necessarily inherent in the cellular complex itself. There may be extrinsic factors involved which have an inhibitory and perhaps only transitory effect on otherwise normal reactions. Some evidence already exists in favour of the presence in malaria of soluble factors which have physiological effects (19). They may well be responsible for the cellular metabolic disturbances and, in fact, constitute the missing link between the parasite and the host.

In the discussion on the behaviour of the blood circulation in malaria we have referred almost exclusively to those local to the liver. It is clear, however, that the local hepatic circulation may also be governed to some extent by what it taking place in the circulation as a whole.

Circulatory failure may develop in acute malaria, usually as a terminal event (1, 21, 22). Study of circulation dynamics in such states has indicated that the condition produced is one of shock, with gross reduction of blood pressures and with limitation of venous return to the heart, associated with

considerable pooling of blood in the peripheral circulation. Contemporaneous with this profound general circulatory disturbance are certain changes in local circulation, particularly notable in the kidney (1, 2). The evidence indicates that when circulatory collapse develops similar local circulatory changes may be initiated also in the liver, with the induction of differential impedence of the centrilobular blood flow. In certain forms of shock, for instance in anaphylactic shock, these centrilobular changes are initiated by active hepatic venous constriction (6). Given time, the circulatory effects, in some instances probably also assisted by cellular swelling or damage, are followed by the appearance of a centrilobular necrotic lesion, which in its various stages is a characteristic feature of the pathological patterns of shock.

The hepatic lesion in malaria thus presents, in part at any rate, either as an isolated response to changes in local blood flow or as a part of more complex vascular responses to general circulatory failure. In this respect, it behaves essentially like the corresponding renal lesion (renal anoxia) associated with anuria and acute uraemia. It clearly represents an example of the progress of factors which are general in character and not specific to the plasmodial infection.

Other circulatory disturbances in other parts of the body occur in malaria which are determined by the intrinsic physiological behaviour of the local vascular endothelium. Injury to the brain vessels, for instance, may lead to increase in endothelial permeability and local loss of fluid with consequent stasis. In the liver these effects do not appear to be important since, as Bollman has shown, the sinusoidal endothelium is normally highly permeable, so that the hepatic cells are bathed in a fluid with a protein content approaching that of plasma.

The final effects of the circulatory phenomena concerned in the genesis of the hepatic lesions in malaria seem also to be governed by other factors, including the prevailing metabolic state of the hepatic parenchymal cells themselves, as indicated by deviations of a general nature in cell function and by changes in basic cellular metabolic activity. The latter, like the circulatory disturbances, appear to be partly specific and partly non-specific. Many undoubtedly belong to the non-specific category and are common to other forms of acute liver damage. Others are dependent in some way, directly or indirectly, on the presence of the malarial infection in the erythrocyte. About these we know very little but there are indications that some of them may be initiated by circulating physiologically active agents and others may depend on secondary effects, for example interference with hormonal balance or production, as is indicated by the stimulating action of cortisone on glycogen formation in the damaged liver in very heavy infections (23, 26).

ALCUNI PROCESSI PATOLOGICI NELLA MALARIA QUALI APPAIONO NELLO SVILUPPO DI LESIONI EPATICHE NELLE INFEZIONI ACUTE

Si può concludere che le lesioni del fegato nella malaria acuta derivano da una combinazione di disturbi cellulari e circolatori. Le alterazioni cellulari epatiche si manifestano con deviazioni della funzionalità del fegato in generale e con disturbi interessanti in modo particolare i sistemi enzimatici mitocondriali. Alcune delle lesioni fisiologiche in questi ultimi sono apparentemente non specifiche in quanto assomigliano a quelle osservate nell'anemia post-emorragica; altre possono essere più specifiche dell'infezione e conducono ad una anossia citotossica, anche in presenza di abbondante ossigeno. Le prime alterazioni metaboliche cellulari non sono in primo tempo dovute ad una mancanza locale di ossigeno, poichè le proprietà dell'emoglobina restano invariate ed il grado di anemia è normalmente inadeguato per produrre tali effetti. D'altra parte si hanno disturbi circolatori che causano un ristagno della corrente sanguigna nell'area centrale di drenaggio dei lobuli epatici. I dati disponibili fanno pensare che questi cambiamenti nella circolazione abbiano un'origine dinamica piuttosto che meccanica, e che possono venire spiegati da una costrizione dell'albero venoso epatico; possono sorgere localmente od in associazione con una deficienza circolatoria generale.

Il completo sviluppo della lesione centrolobulare nel fegato dipende dall'interazione e dalla sommazione dei fattori cellulari e circolatori interessati, più alcuni processi estrinseci o di natura più generale, compresi i disturbi nell'equilibrio ormonico e forse alcune azioni competitive del parassita nei riguardi dell'ospite.

L'inizio di questi processi fa seguito all'invasione dei globuli rossi dell'ospite da parte del parassita. Non si comprende ancora come ciò avvenga, ma i dati che si vanno accumulando tendono a indicare come siano implicate sostanze solubili relativamente semplici. L'identificazione dei legami tra ospite e parassita a questo riguardo rappresenta il problema più importante nelle ricerche fondamentali sulla malaria.

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UNA GRANDE OPERA SOCIALE L'ERADICAZIONE DELLA MALARIA IN ITALIA

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I trattamenti antialate con DDT, praticati dal 1946 al 1953 una volta all'anno, irrorando le pareti interne dei ricoveri dell'uomo e degli animali, hanno portato nella provincia di Latina alla eradicazione della malaria dopo solo tre anni di lotta e alla pratica scomparsa degli anofeli vettori, *A. labranchiae labranchiae* e *A. sacharovi*, rispettivamente dopo due e quattro anni di trattamento.

Seguendo le modalità d'impiego sperimentate a Latina, le operazioni antialate, iniziate quasi generalmente nel 1947 in tutte le altre zone malariche d'Italia, hanno ridotta l'incidenza della malaria praticamente a zero in tutto il territorio nazionale dopo cinque anni di lotta con DDT.

Per quanto riguarda l'anofelismo, i trattamenti antialate hanno portato dopo 4-5 anni di lotta praticamente alla scomparsa degli anofeli vettori dalle zone del Nord e Centro Italia, mentre è residuato nel Sud Italia un certo grado di anofelismo vettore, dato dall'*A labranchiae labranchiae* e dall'*A. superpictus*.

A causa delle vaste inondazioni provocate a scopo bellico, nell'autunno 1943 le aree più basse del litorale tirrenico, dalla Piana di Fondi sino a Maccarese, ritornarono allo stato paludoso. E la malaria, che sino a quel momento era stata validamente contrastata e contenuta, tornò ad imperversare nelle campagne come nei tempi più oscuri del Medio Evo.

Per tale disastrosa situazione, il problema che si imponeva alla Sanità era enorme; il fatto che la malaria era stata sino all'ultima guerra efficacemente controllata, non significava affatto che nelle nuove condizioni ora createsi, si poteva trarne auspicio per una valutazione ottimistica. Si teneva presente l'ondata epidemica, molto meno grave della presente, dovuta alla prima guerra mondiale, che tardò allora a cancellarsi tanto da dover giungere fino al 1936-1937 per riscontrare cifre intorno al livello della malaria raggiunto nel 1914.

Pertanto, il problema di arginare la malaria, che all'indomani della liberazione si poneva sino alle porte di Roma, aveva un carattere d'urgenza, e, allo stesso tempo, urtava contro tali difficoltà da apparire praticamente insolubile.

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Ma la guerra, che è fonte di tanti mali, può anche arrecare qualche vantaggio. Sotto la minaccia della morte, l'uomo agguzza l'ingegno, ed i Governi, sempre tanto parsimoniosi nel sovvenzionare la ricerca scientifica, divengono ad un tratto generosi. E così le scoperte di guerra si succedono: energia atomica, antibiotici e insetticidi di contatto.

Le truppe alleate erano appena giunte nel nostro Paese, e già si parlava di un potente insetticida, chiamato brevemente DDT, nuova arma di lotta contro gli insetti, che era stata spettacolosamente impiegata per eliminare il tifo petecchiale a Napoli nell'inverno 1943-44.

Ma la rivoluzione che veniva portata nella lotta contro le malattie trasmesse dagli insetti a mezzo del DDT era troppo radicale per poter essere prontamente accolta, soprattutto da quei ricercatori che non avevano praticamente svolto campagne contro gli insetti.

In Italia, il Missiroli, che durante 30 anni aveva esplorato tutte le vie per risanare il nostro Paese dalla malaria e sempre aveva raccomandata la lotta contro l'anofele adulto, rigorosamente applicata dai suoi collaboratori nelle zone rurali, fu subito in grado di misurare prontamente i prossimi sviluppi della scoperta del DDT.

Difatti, intuita l'importanza eccezionale che il DDT poteva avere per il risanamento dell'Italia, iniziò all'Istituto Superiore di Sanità febbrili e rigorose ricerche per controllarne l'azione. Assicuratosi delle larghe possibilità del DDT, implicite nella fortunata associazione delle sue due proprietà fondamentali, la sua intensissima azione, efficace ancora con tracce minime e diluizioni estreme, e della sua azione residua anche a notevole distanza di tempo, potè condurre prontamente una sperimentazione nel campo pratico.

Questo primo esperimento venne iniziato il 5 giugno 1945 nella zona Sud-orientale della provincia di Latina, dove la lotta antilarvale con il Verde di Parigi offriva scarse possibilità di successo a cagione dell'estensione della superficie idrica. In questa zona si trova la pianura di Fondi, circondata da estese paludi, ove la popolazione, durante il periodo primavera-estate, vive in capanne costruite con canna palustre, con paglia o con legno. La lotta contro l'insetto adulto in questa zona, che riproduce le condizioni di molte zone rurali dell'Italia del Sud, tendeva a dimostrare che malgrado la popolazione dorma fuori delle capanne durante le caldi notti estive, tuttavia il nuovo insetticida doveva esplicare egualmente la sua efficace azione. Il risultato conseguito confermò la premessa: difatti, con l'irrorazione di DDT delle pareti interne di tutte le case, capanne e ricoveri animali esistenti nella campagna della suddetta zona, si osservò una rapida diminuzione del numero degli anofeli ed il miracoloso arresto nella trasmissione della malaria.

Analoghi risultati erano intanto conseguiti da Soper e Collaboratori nel delta del Tevere ove la popolazione rurale vive invece in buone condizioni di abitabilità.

Da questi primi esperimenti si poteva pertanto trarre la conclusione che

nell'area del Mediterraneo, ove la malaria è in gran parte diffusa da anofeli appartenenti al gruppo dell'*A. maculipennis*, la lotta contro l'insetto adulto è sufficiente a sopprimere la trasmissione della malaria, qualuque siano le condizioni di abitabilità.

Basandosi su questi risultati, Missiroli preparò un piano quinquennale per il risanamento dell'Italia che comunicò il 20 gennaio 1946 in una conferenza tenuta all'Istituto Superiore di Sanità.

Detto piano veniva prontamente approvato dalla Sanità, che attese durante lo stesso anno a predisporre i mezzi necessari per assicurare l'esecuzione dei lavori previsti.

L'approntamento e l'approvvigionamento dei mezzi d'opera, dei trasporti, degli insetticidi e dei materiali vari, la loro distribuzione ed il loro invio ai luoghi d'impiego, l'organizzazione infine di tutti i molteplici servizi connessi con l'esecuzione delle campagne hanno veramente rappresentato ognuno per se, compiti molto difficili e faticosi per la Sanità Pubblica, specie se si tiene conto della vastità del piano di lavoro e delle condizioni dell'Italia in quel momento.

Nel marzo 1946, mentre la Sanità predisponeva i mezzi della attuazione del piano quinquennale, s'iniziò il risanamento della provincia di Latina per trarre criteri pratici per la futura organizzazione della lotta in tutta l'Italia. Collaborarono a questa impresa la Direzione Generale della Sanità che fornì i mezzi, l'Istituto Superiore di Sanità con l'opera di tutto il personale del Laboratorio di Malariologia ed il Comitato Provinciale Antimalarico di Latina con l'opera dei suoi tecnici e disinfestori.

E così, mentre erano ancora aperte e sanguinanti le ferite di una guerra combattuta sul territorio nazionale, mentre ancora le città presentavano le gravi devastazioni di bombardamenti indiscriminati, mentre le campagne stentavano ad essere nuovamente fecondate dal lavoro umano e tutto un popolo, incerto anche sul suo immediato avvenire, soffriva le più gravi conseguenze di una guerra perduta, s'iniziava in Italia una grande opera di redenzione: la liberazione da una secolare nemica del nostro popolo, la malaria.

La lotta con DDT nella provincia di Latina

Dal 1946 al 1951 tutto il territorio malarico della provincia di Latina, compresi i centri urbani e rurali, sono stati sottoposti annualmente ad un trattamento con il DDT, diretto contro l'insetto adulto, irrorando le pareti interne di tutti i ricoveri dell'uomo e degli animali esistenti nella provincia stessa.

Nel 1952 il trattamento antianofelico con DDT venne eseguito soltanto nelle zone rurali con l'esclusione di tutti i centri abitati della provincia, trattamento che venne ripetuto nel 1953 su una zona rurale molto ristretta.

Nel 1954 le operazioni contro le anofeli adulte sono state sospese, mante-

nendo nella provincia soltanto un servizio speciale di sorveglianza sull'anofelismo residuo, diretto dal Centro di Studio per la Lotta contro gli Insetti Nocivi, creato nel 1947 a Latina, dall'Istituto Superiore di Sanità, per potenziare la campagna di eradicazione della malaria in corso.

Durante i primi due anni di lotta venne irrorato il DDT, in forma di soluzioni in petrolio, di emulsioni e di sospensioni in acqua, ottenendo uguale risultato insetticida per intensità e durata.

Pertanto, negli anni successivi è stato possibile sostituire con vantaggio le costose soluzioni di DDT in petrolio con DDT emulsionabile in acqua, e così poter realizzare notevoli economie, sia sul costo del solvente che gravava sul bilancio in modo sensibile per il nostro Paese, importatore di questo prodotto, sia sui trasporti degli uomini e del materiale. Dal 1952 vennero usate esclusivamente paste concentrate di DDT, che danno una sospendibilità in acqua di molto superiore a quella delle emulsioni e delle polveri bagnabili.

E' da tenere ancora presente, che per poter lottare anche contro le mosche domestiche divenute resistenti al DDT dopo il primo anno di applicazione, dal 1948 al 1950 venne impiegato con il DDT anche il Chlordano, limitando però questo trattamento soltanto alle cucine, ingressi di abitazione e ricoveri animali. Divenute nel 1950 le mosche resistenti anche al Chlordano, si sperimentò successivamente, però, senza alcun successo, il Metossicloro (1951) ed il Dieldrin (1952).

La lotta contro le mosche domestiche, divenute resistenti a tutti gl'insetticidi clorurati venne ripresa nel 1954 in tutte le zone rurali della provincia a mezzo di prodotti a base di esteri fosforici, impiegando sino al 1956 sospensioni ottenute da paste polivalenti di DDT e Diazinone (prodotto Geigy) ed in seguito di DDT e Dition (prodotto Montecatini). Come in precedenza con il Chlordano anche con i nuovi prodotti vennero trattate soltanto le cucine, gli ingressi ed i ricoveri animali una volta all'anno.

L'entità degli interventi effettuati durante i primi 5 anni del programma quinquennale di lotta antimalarica e nel 1952, può essere facilmente desunta dalla tabella 1.

TABELLA 1.

Dati relativi all'impiego degli insetticidi di contatto nella Provincia di Latina

Anni	DDT tecnico Kg.	Chlordane tecnico Kg.	Metossicloro tecnico Kg.	Dieldrin tecnico Kg.	Superficie totale trattata m²	Numero degli ambienti trattati	Numero degli abitanti protetti
1946	13.300	—	—	—	7.756.583	139.301	142.426
1947	19.400	—	—	—	10.663.678	198.233	180.264
1948	21.800	1.400	—	—	11.315.242	196.846	179.766
1949	20.700	7.900	—	—	11.564.494	208.518	187.964
1950	20.800	8.500	—	—	13.831.227	264.546	244.815
1951	15.500	—	9.300	—	12.020.120	255.633	191.726
1952	4.900	—	300	1.400	5.698.670	112.519	114.595

Risultati

Morbilità per malaria

Già subito dopo il primo trattamento con DDT (1946) si osservò che la trasmissione della malaria era stata praticamente interrotta in tutta la regione trattata; difatti il numero dei casi di malaria cominciò a decrescere nel giugno 1946, per declinare ancora nell'anno successivo ed arrivare ad una cifra del tutto trascurabile nel 1948 e raggiungere lo zero nel 1949 durante il periodo abitualmente epidemico (Tav. 2). Nelle nostre statistiche si accettarono come casi di malaria tutti quelli accertati clinicamente, senza preoccuparsi del risultato dell'esame microscopico, che avrebbe portato ad una artificiosa riduzione dell'incidenza della malaria.

Tabella 2 — *Morbilità e mortalità per malaria nella provincia di Latina.*

Anni	*N. totale di casi*	*casi primitivi*	*casi di morte*
1944 *a*)	54.929	35.969	120
1945 *b*)	44.712	2.382	160
1946 *c*)	30.929	96	19
1947	6.456	6	0
1948	1.327	0	0
1949	97	0	0
1950 - 1959	0	0	0

a) I dati riguardano soltanto il secondo trimestre dell'anno.
b) Lotta antilarvale con il Verde di Parigi.
c) Primo anno di lotta con il DDT.

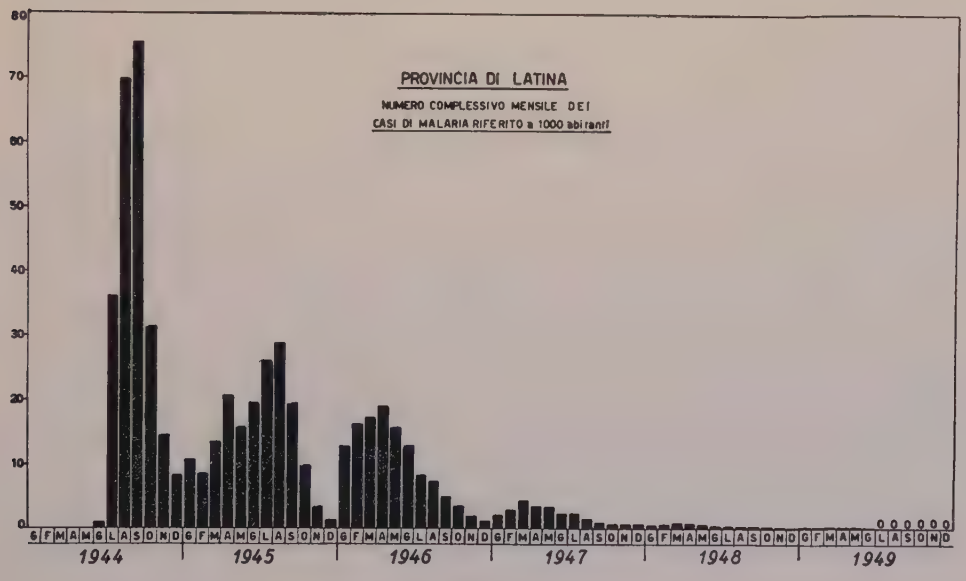

Fig. 1.

La ricerca microscopica, eseguita su gocce spesse di sangue prelevato a pazienti colpiti da malaria che si presentavano agli ambulatori comunali, ha rilevato che l'ultimo caso di terzana maligna si ebbe nel settembre 1947, e nel settembre 1948 quello di terzana benigna. E' da tener presente inoltre, che la profilassi medicamentosa era stata del tutto abbandonata dopo l'impiego del DDT, ed i medicamenti riservati esclusivamente al trattamento dei casi acuti.

Mortalità per malaria

Nel 1946 vennero ancora denunciati alcuni casi di morte per malaria cronica, soprattutto durante il periodo interepidemico; non pervenne invece dopo il primo trattamento con DDT nessuna denunzia di morte per malaria acuta.

Indice splenico e indice parassitario

L'indice splenico che era del 34,2% nel marzo 1946 nella popolazione scolastica sotto i 12 anni di età, è stato riportato a 28,8% nel marzo 1947, a 9,10% nel marzo 1948 ed al 3,0% nel marzo 1951; l'indice parassitario da 10,3% nel marzo 1946 è caduto a 0,49%, a 0,14% e a 0,0% rispettivamente per gli anni 1947, 1948 e 1951.

Anofelismo

Due gli anofeli vettori presenti nella zona, appartenenti al gruppo *maculipennis,* e precisamente l'*A. labranchiae labranchiae* e l'*A. sacharovi.*

Nel 1945, durante la campagna antilarvale con il Verde di Parigi su oltre 100.000 anofeli catturati nel territorio della provincia, il 91,2% appartenevano al gruppo *maculipennis;* dall'esame di 2.168 ovodeposizioni ottenute dagli anofeli del suddetto gruppo risultarono le seguenti percentuali:

A. labranchiae labranchiae	71,6%
A. maculipennis maculipennis	14,3%
A. subalpinus	6,4%
A. melanoon melanoon	4,6%
A. sacharovi	3,1%

Oltre al gruppo *maculipennis* sono presenti nella zona l'*A. claviger* e l'*A. algeriensis.*

Subito dopo il primo trattamento con DDT (1946) si registrò in tutta la regione trattata una diminuzione della popolazione anofelica in una proporzione mai prima osservata con gli altri metodi di lotta usati.

Dopo il secondo trattamento (1947) l'*A. sacharovi* era stato praticamente eliminato dai ricoveri e dai focolai larvali, mentre restavano ancora pochi esemplari di *A. labranchiae labranchiae* nelle stazioni fisse di cattura non trattate, con una densità larvale molto bassa nei focolai corrispondenti.

A potent modern antimalarial
particularly indicated for prophylaxi-

- prompt and sure effect
- low toxicity
- excellent tolerance
- absence of undesirable side-effects
- absence of disagreeable odour and taste
- minimum dosage

ERBAPRELINA

Pyrimethamine

CARLO ERBA MILANO

Dopo il terzo trattamento, (1948) l'*A. sacharovi* era già del tutto assente sia dai ricoveri che dai focolai larvali; era invece ancora possibile trovare qualche raro esemplare di *A. labranchiae labranchiae*, sia allo stato adulto che allo stato larvale.

Dopo il quarto trattamento (1949) si constatò la scomparsa totale anche dell'*A. labranchia labranchiae* dai ricoveri e focolai larvali di tutto il territorio della provincia.

Negli anni successivi non è stato più possibile, malgrado le ricerche più diligenti, di trovare adulti o larve di *A. labranchiae labranchiae* e di *A. sacharovi*, mentre si potevano riscontrare in numerosi focolai un numero alto di larve di tutti gli stadi, soprattutto di *A. maculipennis maculipennis* e di *A. claviger* ed in minor numero di *A. melanoon melanoon* e di *A subalpinus*, e catturare nei ricoveri animali esemplari di *A. maculipennis maculipennis* e di *A. claviger*.

In tutta la provincia non è stata osservata sino ad oggi una diminuzione della sensibilità degli anofeli verso gli insetticidi di contatto.

Eradicazione della malaria dalle altre provincie d'Italia

Il lavoro compiuto a Latina servì di base alla Sanità per la successiva organizzazione della lotta nelle rimanenti provincie malariche d'Italia, ove vivevano circa 10.000.000 di abitanti in zone dichiarate malariche.

Secondo il piano quinquennale, il lavoro s'iniziò nel 1947 in tutte le provincie, ad eccezione di quelle della Sardegna, ove un Ente apposito, l'ERLAAS (Ente Regionale Lotta Anti-Anofelica Sardegna), cercò di realizzare un esperimento di eradicazione degli anofeli, impiegando il DDT sia contro le larve, che contro gli adulti.

I trattamenti antialate furono eseguiti una volta all'anno con sufficiente larghezza di mezzi, seguendo le modalità d'impiego sperimentate nella provincia di Latina.

I risultati di tutto questo lavoro, condotto con intelligenza e dedizione si possono facilmente rilevare dalle tabelle 3 e 4.

La malaria dopo 5 anni d'impiego del DDT è stata praticamente ridotta a zero in tutto il territorio nazionale.

Circa 50 anni fa in Italia la malaria uccideva ancora 5.000 persone ogni anno, cioè circa 50 abitanti su 100.000. Nel 1948, dopo soltanto due anni di lotta antialate con DDT, sono stati segnalati alla Sanità 4 casi di morte per malaria, che però le successive indagini dimostrarono non dovuti a detta malattia. Pertanto, da quando la malaria è stata introdotta in Italia, probabilmente nel terzo secolo avanti Cristo dalla Grecia e dal Nord-Africa, certamente nel 1948, per la prima volta dopo tanti secoli, nessun caso di morte in Italia fu dovuto alla malaria.

TABELLA 3
Casi di malaria segnalati dal 1942 al 1959
(Cifre assolute)

Anno	Morbilità	Mortalità	Anno	Morbilità	Mortalità
1942	104.082	1.075	1951	471	0
1943	37.611 *	—	1952	113	0
1944	373.491	421	1953	19	0
1945	411..602	386	1954	15	0
1946	374.163	280	1955	21	0
1947	210.828	93	1956	91	0
1948	92.327	4	1957	40	0
1949	19.462	0	1958	17	0
1950	3.507	0	1959	16	0

(*) Dati incompleti a causa delle operazioni belliche.

TABELLA 4.
Casi di malaria segnalati dal 1952 al 1959

Anno	Primitivi autoctoni	Recidivi	Importanti dall'estero	Totale
1952	43	60	10	113
1953	4	10	5	19
1954	4	6	5	15
1955	2	10	9	21
1956	78	6	7	91
1957	1	36	3	40
1958	2	10	5	17

Riguardo all'*anofelismo residuo*, mentre nelle province del Nord e Centro Italia la lotta antianofelica ha portato praticamente alla scomparsa degli anofeli vettori, nel Sud Italia si può invece rilevare ancora oggi un certo grado di anofelismo vettore, dato da *A. labranchiae labranchiae* e *A. superpictus*.

Pertanto non si può certamente escludere qualche piccolo episodio di ripresa della trasmissione della malaria nell'Italia meridionale, specie in Sicilia, ma dato che le sorgenti di infezione sono scomparse o estremamente ridotte, gli eventuali episodi potranno essere facilmente circoscritti e controllati da un adeguato servizio di sorveglianza, come praticamente si è verificato durante la lieve ondata epidemica (78 casi primitivi) avuta in un centro rurale della Sicilia nel 1956.

Per quanto riguarda la resistenza degli anofeli verso gli insetticidi di contatto, i risultati ottenuti dalle esperienze condotte sul campo, sia dall'Istituto di Malariologia E. Marchiafava, in numerose zone dell'Italia continentale, sia dal-

l'Istituto d'Igiene dell'Università di Palermo in zone trattate della Sicilia, sia dal Centro di Studi per la Lotta contro gli Insetti Nocivi dell'Istituto Superiore di Sanità nella provincia di Latina, non hanno dimostrato una diminuzione della sensibilità degli anofeli verso gli insetticidi impiegati, anche in zone trattate con DDT per 10 anni.

Costo della lotta anti-anofelica

Le spese sostenute dalla Sanità per le campagne antianofeliche dal 1947 al 1952, escluse quelle della Sardegna, ammontarono a 6.933.197.629 Lire, con una media annua di circa 1.250.000.000 Lire, corrispondenti a circa 250 Lire per abitante delle zone trattate.

Calcolando che la mercede giornaliera di un operaio è di circa 1.200 Lire, si è stati in grado di difenderlo dalla malaria e da tutte le altre malattie trasmesse dagli insetti domestici con una somma equivalente al suo guadagno di circa un'ora di lavoro.

A GREAT SOCIAL ACHIEVEMENT: THE ERADICATION OF MALARIA IN ITALY.

From the results of two antianopheline campagns conducted in Italy in 1945 (one by Missiroli and coworkers in the « Paludi Pontine », and the other by Soper and coworkers in the « Agro Romano ») it could be concluded that in the Mediterranean area, in which malaria is due in great part to anophelines of the *A. maculipennis* group, adulticide measures were sufficient to interrupt the transmission of malaria whatsoever were the housing conditions.

Following Missiroli's five-year antimalarial plan for Italy proposed in January 1946, operations for the eradication of malaria in the Province of Latina were initiated in March of the same year, in order to obtain new pratical criteria for the future organization of the antimalarial campaign in the whole of Italy.

On basis of the previous experience with DDT in 1945 chemotherapeutic prophilaxis in the Province was discontinued and antimalarial drugs were reserved exclusively for treatment of acute cases. In the same way the antilarval treatment and mechanical protection were left ont.

Anti-adult operations with DDT were carried out once from 1946 to 1953: the internal walls of all human dwellings and animal quarters in the malarious zone of the Province were sprayed. A vigorous surveillance on residual anophelism was maintained thence.

It has to be mentioned that from 1954 onwards there were still used insecticidal formulations on basic of DDT and Diazinon (Geigy) and DDT and Dithion (Montecatini) in quite a large measure for combating houseflies resistant to chlorinated hydrocarbons. These treatments were restricted however, to certain parts of the houses (kitchens and barns).

Anti-adult measures with DDT have brought about eradication of malaria (*P. falciparum* in the third year and *P. vivax* in the fourth year of the campaign).

As regards the malaria vectors, through treatment with DDT *A. sacharovi* has disappeared from the Province after 2 years and *A. labranchiae labranchiae* after 4 years of the campaign.

In the other provinces of Italy in which there are zones considered malarious (with exception of Sardinia, where it was tried to achieve eradication of anoprelines), the anti-adult measures with DDT, started nearly all in spring 1947, following the procedures used at Latina, have practically led to disappearance of malaria after 5 years. After so many centuries of the ravages of malaria, already in 1948, i.e. only two years after the start of the DDT anti-adult campaign, no case of death due to malaria was reported in Italy.

In northern and Central Italy the anti-adult campaign has pratically led within 4-5 years to disappearance of the anophelines, while in Southern Italy a certain amount of malarious vectors (*A. labranchiae* and *A. superpictus*) exist still today.

The possibiiity of some small episodes of renewed malaria transmission by this residual anophelism can not be excluded, but since the infection sources, if not completely absent are very strongly reduced, such episodes should be easily circumscribed and well controlled through an adequate surveillance service. This was so in practice during the brief and localized epidemic in 1956 in a rural centre in Sicily.

As regards resistance of the anophelines towards contact insecticides, the results obtained by different workes have not revealed so far a decrease in sensibility of Italian anophelines against the insecticides in use.

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LA RICERCA DEI CASI NEL CORSO DELL'ERADICAZIONE DELLA MALATTIA

EMILIO PAMPANA

Per la prima volta è ora possibile di fare una valutazione preliminare dei vari metodi di ricerca dei casi di malaria nella fase di consolidamento di programmi di eradicazione. Dai dati disponibili per 14 paesi sembra doversi concludere che ciascuno dei tre metodi di ricerca, attiva, passiva, e mediante rilievi parassitari (« surveys ») riesce a trovare dei casi che molto verosimilmente erano sfuggiti agli altri. Onde l'importanza di utilizzare in questa fase di ogni programma tanto la ricerca attiva quanto la passiva e di non rinunciare, in determinate circostanze che vengono suggerite, neppure ai « surveys » parassitari che possono ancora contribuire a scovare dei casi, inclusi quelli consistenti in parassitemie asintomatiche.

L'eradicazione della malaria si basa su due tecniche fondamentali di sanità pubblica che si sono generalizzate in questi ultimi anni.

L'una, generalmente impiegata durante la fase di « attacco » (1) è la spruzzatura di insetticidi ad azione residua sulle pareti interne di tutte le case, nessuna esclusa, della zona malarica, secondo il metodo che in inglese significa « di copertura totale »; l'altra, la « sorveglianza epidemiologica » consiste nello identificare tutti i casi (2) di malaria e nel renderli innocui. In quest'articolo ci limiteremo ad alcune considerazioni sull'identificazione, la ricerca dei casi (« detection » in inglese e « dépistage » in francese) omettendo di trattare l'investigazione epidemiologica necessaria per tutti i casi confermati positivi, nonchè le misure da applicare, sia ai sospetti, sia ai casi comprovati.

Eradicazione della malaria significa, come è noto, l'eliminazione delle sor-

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(1) Ricorderemo che il Comitato di Esperti della Malaria dell'Organizzazione Mondiale della Sanità (OMS) distingue quattro fasi successive in un programma di eradicazione: quella della preparazione, dell'attacco, del consolidamento e del mantenimento dell'eradicazione avvenuta. World Health Organization, Expert Committee on Malaria, Sixth Report (1957) *Wld Hlth Org. techn, Rep. Ser.*, 123.

(2) In questo articolo la parola « caso » indica una persona con parassiti malarici nel sangue sia ch'ella abbia o non abbia, febbre o altri sintomi.

genti d'infezione malarica e non già quella degli anofeli vettori. In certi casi però, se la specie vettrice è sufficientemente antropofila ed endofila gli insetticidi sembrano farla sparire. Questo fenomeno, che ovviamente preclude ogni trasmissione, parrebbe render superflua la ricerca dei casi per suggerire invece la creazione di un servizio di segnalazione immediata di un'eventuale ricomparsa dei vettori. Ma probabilmente esso sarebbe più difficile e non meno costoso del servizio di ricerca dei casi; ed è infatti su questa che si basa pure la sorveglianza epidemiologica anche nelle zone che son rimaste prive di vettori.

Per la ricerca dei casi la rilevazione episodica della parassitemia in un gruppo a caso della popolazione, il classico « survey » parassitario, specialmente dei bambini, non basta, poichè esso non potrebbe trovare che i casi compresi nel gruppo esaminato e nel solo giorno dell'esame.

La vera ricerca dei casi si distingue, secondo la maniera in cui viene fatta, in passiva o attiva. Nella ricerca passiva è il soggetto, sospetto di malaria o febbricitante, che va dalla persona designata a prelevare strisci di sangue e distribuire l'antimalarico; nella ricerca attiva invece speciali agenti di sorveglianza visitano periodicamente ogni località, ogni villaggio, spesso casa per casa, ricercandovi casi di febbre, in atto o recenti, onde prender loro strisci di sangue e somministrare una dose unica di antimalarici, dose che, come nel caso della ricerca passiva, oltre che giovare clinicamente al soggetto, qualora egli fosse veramente un malarico, dovrebbe anche sopprimer subito il pericolo ch'egli potesse trasmetter l'infezione alla zanzara. Ma siccome la ricerca attiva, per bene che sia fatta, non potrà praticamente effettuarsi giornalmente a tutta la popolazione, è evidente che un certo numero di casi sfuggirà, o verrà trovato tardivamente. Supponendo che le visite vengano fatte mensilmente, come è regola in vari paesi, possiamo imaginare che se un caso di *vivax* si produce all'indomani della visita dell'agente di sorveglianza, non solo dei casi secondari potrebbero prodursi prima della visita seguente, ma sarebbe anche possibile che al momento di questa vi fossero degli anofeli già infettati su tali casi (1) sì che nel mese successivo essi potrebbero distribuire numerose infezioni, a meno che la località non fosse stata spruzzata. E' quindi evidente che anche la ricerca attiva fatta coscienziosamente e mensilmente non è soddisfacente e che un meccanismo di ricerca continuativa e non soltanto periodica è altamente desiderabile. La ricerca attiva dovrebbe quindi essere integrata dalla ricerca passiva. (2)

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(1) Ciò è possibile ammettendo che la temperatura media sia di 23°C o superiore e che per conseguenza il ciclo estrinseco non duri più di 12 giorni; che l'incubazione sia di altri 12 giorni e che i gametociti compaiano nel sangue dei casi secondari lo stesso giorno, o uno o due giorni dopo, la comparsa dei primi parassiti asessuati.

(2) Vedasi a questo proposito anche il Settimo Rapporto del Comitato di Esperti della Malaria dell'OMS: (1959) *Wld Hlth Org. techn. Rep. Ser.*, 162.

In condizioni particolarmente favorevoli è lecito supporre che la ricerca passiva, da sola, potrebbe essere la soluzione ideale. Con la collaborazione dei medici, degli istituti e dispensari sanitari, e con quella di « collaboratori volontari » residenti in ogni località si potrebbe veramente sperare che tutti i casi febbrili si presentino al personale incaricato della ricerca. Inoltre la ricerca passiva costa poco, quella attiva è molto costosa. Ma le condizioni che assicurano efficacia al metodo passivo mediante i collaboratori volontari non possono purtroppo verificarsi in molti paesi, poichè esse consistono nell'esistenza di un adeguato servizio sanitario rurale, nell'aver fatto un'intensa e fruttuosa opera di educazione sanitaria di tutta la popolazione in materia di eradicazione della malaria, e, finalmente, nel disporre del numero adatto di collaboratori volontari, coscienziosi, efficienti ed entusiasti, situati in località facilmente accessibili da ogni dove.

* * *

In questi ultimi anni molto si è speculato sulle tecniche della sorveglianza epidemiologica ed è soltanto ora che si può forse cominciare a valutarle, poichè possiamo disporre almeno dei resultati preliminari da vari paesi. Nello scorso maggio la XI Assemblea Mondiale della Sanità ebbe un imponente rapporto sullo sviluppo dell'eradicazione della malaria nel mondo (1). Da esso risulta che alla fine del 1958 operazioni di sorveglianza in fase di consolidamento erano intraprese da almeno 26 paesi; e da molti di più se vi aggiungessimo quelli dove la sorveglianza è in atto sebbene la zona si trovi ancora in fase di attacco, sia cioè soggetta a misure insetticide di copertura totale. Sembra ovvio che per valutare il rendimento di una particolare tecnica di ricerca dei casi sia specialmente indicata la fase di consolidamento che permette la moltiplicazione dei casi da ogni infezione sfuggita al controllo. Abbiamo esposto nella Tavola alcune delle informazioni contenute nel rapporto citato, limitatamente a quei paesi per cui il rapporto fornisce dati relativi ad aree di consolidamento e dove vige la ricerca attiva, sola o accompagnata da ricerca passiva, dei casi. In base a tale cernita abbiamo escluso dalla tavola informazioni su programmi altrimenti di grande interesse, sì che dei 26 paesi soltanto 14 regioni in diversi paesi e territori, con una popolazione complessiva di 17,3 milioni figurano nella Tavola.

Vediamo anzitutto, dalle colonne 6 e 7 che il rendimento delle denuncie da parte di medici ed ospedali è generalmente tanto scarso da essere insignificante. Soltanto in Irak, forse a seguito dell'emozione e della morbidità cau-

(1) WHO, *Report on development of malaria eradication programme*. Documento ciclostilato, A12/P&B/10, 4 May 1959.

AREE, DI VARI PAESI, IN FASE DI CONSOLIDAMENTO IN 1958, CON RICERCA ATTIVA DEI CASI

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	PAESE	Area in fase di consolidamento	Popolazione dell'area in fase di consolidamento	Frequenze con cui l'ag. di sorv. (ricerca attiva) visita i villaggi e le case	Numero di abitanti cui deve accudire ogni agente di sorveglianza	Denuncie Casi denunciati da Medici, osped. e cliniche		"SURVEYS" PARASSITARI			RICERCA ATTIVA dei casi mediante agenti di sorveglianza				Ricerca passiva dei casi mediante							Totale positivi
															Collab. volontari		vetrini inviati da dispensari rurali, osped. e Ist. San			Vetrine inv. da al. fonti		
		K m²	Migliaia			Vetrini esaminati	Positivi	Vetrini esaminati	Positivi	% positivi	Vetrini esaminati	Positivi	% positivi	Col.11 / Col. 3 x 100	Vetrini esamin.	positivi	Vetrini esamin.	Positivi	% Positivi	Vetrini esamin.	Positivi	
1	*Argentina*	23100	743	1 mese		37	11				12347	14	0,11	1,7	612	0						25
2	*Ceylon*	5180	2478	irregol		135	?				1037	54	5.3	0,04			14009	53	0,38			107
3	*Filippine*	110977	5466	3 settim	15000			606573*	1097*	0,17	156451**	6957**	4,4**	2,8**								
4	*Guadeloupe*	752	129	nei villag. 6 mesi	15000						4877	0	0	3,8			842	0				0
5	*India*	12316	1322	2-4 sett.	5430						22696	30	0,13	1,7								30
6	*Irak*	61351	1253	1 mese		398	22	8627	31	0,36	140460	109	0,07	11								162
7	*Iran*	?	2449	1 mese	8300						76258	144	0,19	3,1								144
8	*Messico*	36790	59	1-3mesi	5000						2652	0	0	4,5	652	0	983	0		162	0	0
9	*Perù* (occ.)	5110	14	2 mesi	25000						669	3	0,45	4,7								3
10	*R.A.U.* (Siria)	1500	126	1 mese	15000 / 20000			15598	116	0,74	12656	0	0	13								116
11	*Swaziland*	1200	125	2 settim.	4000	23	0				2083	15	0,73	1,7								15
12	*Trinidad*	26	160	2-4 sett.	5000	2	2				19827	0	0	12								2
13	*Turchia*	?	2294	2 settim.	5500			72743	88	0,12	64561	1357	2,1	2,8								1445
14	*Venezuela*	43712	703			16	0				63110	38	0,06	9,-			6488	8	0,12			46
	Totali e medie		1732								579694	8721	*1,54*	*3,34*								

(*) Cifre per il 1957. (**) Cifre risultanti dalla ricerca attiva e passiva insieme per il periodo luglio-dicembre 1958.

sata dalla resistenza al DDT dell'*Anopheles stephensi* i vetrini mandati dai medici e dagli ospedali assommarono a qualche centinaio arrivando a rappresentare quasi il 14% del totale dei positivi. Sembra che anche dove la denuncia della malaria è obbligatoria i medici prendano e mandino strisci solo dei casi che clinicamente sono di malaria; così si spiegherebbe l'alta positività dei pochi vetrini dell'Argentina e di Trinidad (Notisi che nei tre paesi qui citati la denuncia è obbligatoria; dei paesi menzionati nella colonna 6 essa non è obbligatoria né a Ceylon né nello Swaziland).

La ricerca attiva (colonne 11-14) non svelò nessun positivo in ben 4 programmi (col. 12). Negli altri la percentuale di positività variò dal 0,06% del Venezuela al 5,3% di Ceylon: media 1,54% con una mediana a 0,12%. Se escludiamo dal calcolo i quattro paesi dove la ricerca attiva dette zero come resultato, allora la media diventa 1,62% e la mediana 0,32% (1).

Tali constatazioni non mancano di utilità poichè esse ci suggeriscono la percentuale di positività che potremmo aspettarci in fase di consolidamento fra tutti i vetrini prelevati. A suggerirci, d'altra parte, l'ordine di grandezza del lavoro che avrebbero i microscopisti viene la colonna 14, che abbiamo preparato per mostrare il numero di vetrini prelevati dalla ricerca attiva in fase di consolidamento come percentuale della popolazione dell'area relativa. Tale percentuale è di 3,34%; gli estremi 0,04% e 13%, la mediana essendo di 3,45%. Tali valori, in altre parole, esprimono il numero di soggetti con febbre in atto o febbre recente o altrimenti sospetti di malaria che il meccanismo di sorveglianza è riuscito a trovare e di cui ha mandato preparati di sangue ai laboratori.

Disgraziatamente, nella Tavola, la ricerca passiva mediante collaboratori volontari non figura che in una zona dell'Argentina e in una del Messico, ambedue con un numero troppo esiguo di vetrini e con nessuno positivo. Quella altra forma di ricerca passiva, che non dovrebbe mai mancare in qualunque schema di sorveglianza, quella basata sull'invio, da parte dei dispensari o degli ospedali rurali, di vetrini di casi febbrili o comunque sospetti, è stata negativa (col. 17 e 18) in due paesi dove anche la ricerca attiva lo era stata (Guadalupa e Messico). Ma questa sola forma di ricerca passiva può dare percentuali più alte di positività di quelle della ricerca attiva: così in Venezuela la positività della prima era doppia di quella della seconda, 0,12% contro 0,06%; mentre a Ceylon la positività di tale ricerca passiva era quasi 14 volte minore benchè, avendo esaminato un numero corrispondente più grande di strisci, fosse riuscita a trovare 53 positivi contro 54 della ricerca attiva. Anche questi

(1) Da questa ultima serie potremmo anche escludere i valori delle Filippine, in quanto si riferiscono solo a sei mesi e includono anche la ricerca passiva; e quelli di Ceylon, per ragioni inerenti al metodo seguito e principalmente per l'esiguo numero di vetrini. Così facendo la positività scenderebbe a 0,31% e la mediana a 0,16%.

pochi dati (1) ci sembrano confermare la necessità che la sorveglianza attiva sia sempre accompagnata dalla ricerca passiva, almeno di quella che possono fornire le istituzioni sanitarie urbane e principalmente rurali, rammaricandoci che quella più capillare, mediante i collaboratori volontari sia, a detta di varie autorità, di ben difficile applicazione in molti paesi.

Diamo ora uno sguardo ai resultati delle rilevazioni parassitarie in massa, per le quali preferisco usare il termine inglese di « surveys » parassitari (col. 3, 10). Vennero fatti in quattro paesi e in ogni paese svelarono dei casi positivi. Anzi, nel caso della Siria svelarono 116 casi, mentre la sorveglianza attiva non ne aveva trovato nessuno su un numero totale di vetrini dello stesso ordine di grandezza, circa il 13% della popolazione (in fase di consolidamento). Negli altri tre paesi il numero di casi positivi trovati dai « surveys » sono stati minori di quello della ricerca attiva, benchè nel caso dell'Irak il quoziente di positività dei primi fosse più alto che quello della ricerca attiva. Comunque i « surveys » scoprirono, in Irak 31 casi, in Turchia 88 e nelle Filippine (dati per il 1957) 1097 casi positivi.

Viene quindi fatto di domandarci se il vecchio metodo del survey parassitario debba davvero abolirsi nella fase di consolidamento come da molti si vorrebbe. E' certo che quando la trasmissione si avvicina a zero essi svelano pochissimi casi; ma se alcuni ne vengono svelati, siccome in fase di consolidamento è necessario trovarli teoricamente tutti, come potremmo consigliare di rinunciare a tali metodi di ricerca prima che essi si siano mostrati incapaci di svelare dei casi? Riconosciamo che in certi « surveys », come in Turchia o nelle Filippine, occorrerà esaminare 500-800 gocce spesse prima di trovarne una positiva; ma forse che la ricerca attiva non offre simili difficoltà, quando vediamo che nel Venezuela occorreva esaminarne ben 1600 prima di trovarne

(1) Chi volesse dare esempi di percentuali di positività più alte nella ricerca passiva li troverebbe facilmente in vari paesi latino-americani dove essa gode dell'enorme ausilio dei collaboratori volontari. Ne diamo alcuni benchè dovendo citare cifre riferentisi a paesi o aree in fasi diverse da quelle di consolidamento.

	Ricerca passiva mediante collaboratori volontari			Ricerca attiva		
	Vetrini esaminati	Positivi	% positivi	Vetrini esaminati	Positivi	% positivi
Argentina (tutto il paese con aree di malaria eradicata, altre in attacco, altre in consolidamento)	4.458	216	4,7%	33.978	629	1,8
Costarica (in fase di attacco)	2.310	313	13,5	32.021	1.063	3,4
Guatemala (in fase di attacco)	3.329	448	13,4	13.903	876	6,3

una positiva? Eppure nessuno vorrebbe oggi suggerire di rinunciare alla sorveglianza se si vuole giungere all'eradicazione.

Il criterio di valorizzare il metodo che dà la più alta positività per trascurare quelli che sono meno redditizi è molto pericoloso. Dovremmo allora consigliare che oltre alla ricerca attiva e passiva dei casi, si debbano anche fare sistematicamente dei « surveys » su tutta la popolazione? Ricordiamo intanto che essi non sarebbero mai esami di tutta la popolazione, ma l'esame di campioni rappresentativi, generalmente limitati a gruppi di bambini sotto i dieci anni di età e a numeri che dovrebbero esser calcolati statisticamente tenendo conto della grande presunta rarità dei parassitiferi. Ammesso che i « surveys » possono anch'essi trovare dei casi, sarebbe troppo facile rispondere che essi dovrebbero esser fatti su base nazionale, magari più volte all'anno, fino ad eradicazione dichiarata, poichè esistono dei limiti alle possibilità pratiche. Ci sembra però che essi sarebbero particolarmente raccomandabili nelle seguenti circostanze:

1) alla fine della stagione malarica dell'anno che, secondo il piano, dovrebbe esser l'ultimo della fase di attacco; tali surveys parassitari dovrebbero abbracciare tutta la regione dove le spruzzature non sono previste per l'anno seguente;

2) durante la fase di consolidamento, limitatamente alla popolazione, di qualunque età, di particolari località, o gruppi di località dove la sorveglianza abbia trovato casi positivi ed autoctoni; o dove, eventuali surveys dell'anno precedente, abbiano trovato dei casi positivi non previamente identificati dalla sorveglianza. Questi rilievi parassitari dovrebbero essere estesi possibilmente a tutta la popolazione.

E' forse superfluo notare che questi « surveys » non vengono consigliati per arrivare a determinare un indice parassitario, che in ogni caso dovrebbe esser vicino a zero; ma per identificare casi positivi in aggiunta a quelli trovati dalla sorveglianza. Se questa era fatta bene, i casi rivelati dai surveys consisteranno probabilmente di parassitiferi sani, senza sintomi e potranno rappresentare le sorgenti d'infezioni che sono stati l'origine dei casi eventualmente scoperti dalla sorveglianza.

In conclusione, nella fase di consolidamento, nessuno sforzo dovrebbe esser di troppo, nessuna tecnica dovrebbe esser trascurata, per trovare per quanto sia possibile tutti i casi. Sappiamo dagli studi di Macdonald (1) quanto siano pericolose poche sorgenti d'infezione in questa fase. E citeremo Bastianelli e Bignami (2) che già sessant'anni fa notavano che « una sola zanzara, pungendo più volte, può infettare vari uomini ».

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(1) MacDonald G. (1957): The epidemiology and Control of Malaria, London, Oxford University press, pp. 201.

(2) Bastianelli G. e Bignami A., La malaria e le zanzare, comunicazione fatta al X congresso della Società Italiana di Medicina interna, Seduta del 26 ottobre 1899.

THE DETECTION OF MALARIA CASES DURING THE ERADICATION OF THE DISEASE

For the first time it is now possible to attempt a preliminary evaluation of the various methods used for the detection of malaria cases during the consolidation period of a malaria eradication programme. Information in now available from fourteen countries and it points out that every one of the three methods of detection, active and passive method, as well as the parasite survey, succeeds in tracing cases that very likely had been missed by the others. It is therefore important to utilize in the consolidation phase of every programme both active and passive detection and even in particular circumstances, the parasite surveys, as they may detect further cases, including asymptomatic parasitaemias.

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CONSIDERAZIONI GENERALI SOPRA IL PIANO DI LOTTA CONTRO LA MALARIA, NELL'AMAZZONIA BRASILIANA, COL METODO DEL SALE CLOROCHINATO

Mario Pinotti (*)

Vengono illustrati i problemi epidemiologici generali e speciali relativi alla eradicazione della malaria in Brasile. Nella regione amazzonica, in aggiunta a questi, se ne hanno altri particolari quali l'immensità del territorio, la rarefazione della popolazione, e la scarsezza od assenza dei mezzi di comunicazione e di trasporto. Il metodo del sale clorochinato è appunto sorto come tentativo di soluzione del problema dell'Amazzonia; dopo l'esposizione di alcuni recenti risultati ottenuti con esso, sono illustrati i principali problemi connessi con l'applicazione di tale metodo nell'Amazzonia Brasiliana.

1. Uso degli insetticidi ad azione residua nella lotta contro la malaria.

Le autorità brasiliane, già da molto tempo stanno facendo grandi sforzi per combattere la malaria nel Brasile. Però, la possibilità di una lotta sistematica contro questa malattia endemica, nell'ambito nazionale, è sorta soltanto dopo l'avvento degli insetticidi ad azione residua, come il DDT, il cui uso in larga scala ha avuto inizio nel 1947.

Il programma di diditizzazione delle abitazioni è condotto dalle autorità sanitarie brasiliane con risultati magnifici. L'incidenza della malaria in Brasile, che ammontava nel 1940 per lo meno a 6 milioni di casi, è scesa nel 1958 a 250 mila casi.

L'applicazione periodica e sistematica del DDT in alcune zone infestate riuscì anche, come venne osservato, ad eliminare completamente l'endemia.

Nell'anno in corso, il Governo Brasiliano ha iniziato un grande programma di eradicazione della malaria che otterrà un valido aiuto finanziario dal Governo degli Stati Uniti dell'America del Nord (Punto IV), come pure la cooperazione della « Organizzazione Panamericana di Sanità » (OPAS).

L'esecuzione di questo importante lavoro è affidata alla « Campagna per l'Eradicazione della Malaria », del Ministero della Sanità.

2. Problemi speciali relativi alla malaria.

Come è stato riferito, con il programma di controllo basato sulle applicazioni a domicilio del DDT, l'incidenza della malaria nel Brasile ha avuto una forte diminuzione. Con la Campagna di eradicazione della malaria, ora ingaggiata, il DDT dovrà da solo eliminare completamente la trasmissione ancora esistente nella maggior parte degli Stati, fino a che le specie portatrici della malaria del Brasile non acquistino una resistenza a questo insetticida o ai suoi similari.

Ci sono, tuttavia, in certe regioni del Paese, problemi epidemiologici speciali che, pur in assenza dei fenomeni di resistenza, potranno impedire la vittoria completa del DDT o degli insetticidi ad azione residua; potranno cioè impedire che la malaria possa essere definitivamente eradicata, con questo solo mezzo profilattico (1). Tali problemi sono, in succinto, i seguenti:

2.1. Possibilità di trasmissione della malaria al di fuori delle abitazioni, per mezzo di certe specie vettrici (*A. cruzii cruzii. A. bellator* e *A. darlingi*). Negli ambienti dove questo problema si presenta, anche se il grosso della trasmissione sarà eliminato con l'applicazione di insetticidi nelle case, potrà rimanere una certa trasmissione residua, non attaccata dall'insetticida.

2.2. Esistenza di abitazioni che non possono essere adeguatamente protette dagli insetticidi (mancanza di pareti) rimanendone, di conseguenza, gli abitanti esposti alla trasmissione della malaria.

2.3. Nomadismo e seminomadismo di certi gruppi (raccoglitori di caucciù, cercatori d'oro e di pietre preziose, taglialegna) che dovendosi spostare continuamente, vivono in abitazioni provvisorie, il che impedisce od ostacola una protezione adeguata per mezzo degl'insetticidi ad azione residua.

Con lo sviluppo della Campagna di Eradicazione della Malaria, focolai circoscritti di malaria parzialmente refrattari agli insetticidi e motivati da uno o più dei fattori epidemiologici sopra numerati, potranno sorgere in diversi punti della zona endemica, con trasmissione da *A. cruzii cruzii, A. bellator* o *A. darlingi.*

Non dovranno però acquisire grande importanza, dal momento che potranno esser eliminati da medicamenti specifici soppressivi o profilattici sia col metodo classico della loro distribuzione periodica in compresse di casa in casa, sia mescolandoli al sale da cucina, metodo profilattico creato e sviluppato in Brasile ed oggetto principale di questa pubblicazione.

3. Il problema della malaria nella regione amazzonica.

La regione amazzonica costituisce al momento per la Campagna di Eradicazione della malaria nel Brasile il maggior problema da affrontare. In questa area, in cui il principale vettore è l'*A. darlingi,* oltre a tutti i problemi di

carattere epidemiologico già riferiti e che, per se stessi, impediscono agli insetticidi di dare risultati completi, sono presenti altri seri problemi di ordine amministrativo che costituiscono un difficile ostacolo a un programma di eradicazione della malaria basato sull'uso domestico di insetticidi.

L'Amazzonia Geografica (Stati del Parà, delle Amazzoni, e Territori Federali dell'Acre, Amapà, Rondônia e Rio Branco) abbraccia un'area di 3.566.734 Km^2, con una densità demografica di appena 0,65 abitanti per Km^2. I mezzi di comunicazione e di trasporto sono scarsi o addirittura inesistenti, rendendo l'accesso alle case di determinate regioni quasi impraticabile. Per la copertura totale di questa area con un programma di diditizzazione domiciliare sarebbe necessaria la mobilitazione di un gran numero di persone, di una vasta rete di mezzi di trasporto e di una complessa organizzazione amministrativa. A causa di tutti questi fattori un programma integrale di eradicazione della malaria nell'Amazzonia, con insetticidi ad azione residua costituirebbe una operazione difficilissima, di durata indefinita, di costo elevatissimo e d'esito molto dubbio.

4. Metodo del sale clorochinato.

Il metodo del sale clorochinato è sorto come un tentativo di soluzione del problema dell'Amazzonia. Si tratta di un metodo profilattico medicamentoso — associazione del chinino al sale di cucina — che è già universalmente noto e la cui efficacia è stata comprovata in varie zone malariche del Brasile (2 - 3).

La clorochina è addizionata al sale nella proporzione di 30 mg. di base per 10 g. di sale. Considerando che un adulto ingerisce in media da 10 a 15 g. di sale al giorno, la dose di clorochina sarà da 210 a 315 mg. per settimana, dose considerata efficace per eliminare il parassita dal sangue e innocua all'uomo. La clorochina non provoca nessuna alterazione nell'aspetto e nel sapore del sale, il che facilita la sua accettazione da parte del pubblico consumatore.

Spetterà alla Campagna di Eradicazione della malaria di provvedere a miscelare il chinino con il sale, all'origine, e a controllare, nella maniera più perfetta possibile, tutto il sale che entra nelle zone protette.

Nella Regione Amazzonica esiste una certa facilità di controllo del sale e della sua clorochinizzazione, dal momento che la grande maggioranza del prodotto è importata sotto la specie di sale grosso, a granuli, attraverso la foce del Rio delle Amazzoni, e macinato nei grandi magazzini di Belém e Manàus, dove viene insaccato per la vendita nell'interno. In questi magazzini sarà fatta l'aggiunta del medicamento al sale prima dell'insaccamento del prodotto stesso.

La clorochina, introdotta con il sale di cucina, anche se non distrugge il parassita nei tessuti, lo elimina dal sangue periferico, sopprimendo così le manifestazioni cliniche della malattia, ed impedisce inoltre il contagio dell'in-

setto vettore. Teoricamente quindi, se il contagio fosse interrotto durante il periodo di quattro anni — tempo sufficiente alla distruzione naturale del parassita nell'organismo degli individui antecedentemente infetti — la malaria sarebbe estirpata e l'uso del sale clorochinato potrebbe essere sospeso.

Particolari sopra la stabilità della clorochina nel sale, la sua innocuità per l'uomo, la sua eliminazione attraverso i liquidi organici ecc., sono stati oggetto di varie pubblicazioni di autori brasiliani (2, 3, 4, 5). Altri lavori dello stesso gruppo di autori hanno messo in rilievo i risultati ottenuti col metodo del sale clorochinato in ammalati degenti negli ospedali e in osservazioni di campagna (6 e 7). Alcuni risultati più recenti ottenuti col sale clorochinato in varie regioni del Brasile sono qui sotto riassunti.

4.1. *Territorio federale dell'Amapà.* — In una grande zona di contagio da *A. darlingi* si verificò, in questo territorio, nel primo semestre del 1957, che, con appena un mese di uso del sale clorochinato, l'indice parassitario ebbe una caduta sorprendente dal 60,8% al 2%.

4.2. *Area dell'ingegnere Delabela* (Stato di Minas Gerais). Questa zona in cui il responsabile del contagio è l'*A. darlingi,* è notoriamente un focolaio di malaria in parte refrattario al DDT, per cui permane un certo contagio residuo, anche dopo tre applicazioni di DDT per anno (1 e 8).

Di fronte all'impossibilità di eliminare completamente la trasmissione per mezzo degli insetticidi, si decise di impiegare il metodo del sale antimalarico, essendo il DDT ivi usato per l'ultima volta, nell'aprile del 1956.

Vediamo attraverso la Tabella 1, alcuni dati sull'uso del sale clorochinato in questa zona.

Nel periodo 1950 - 1954 i casi di malaria registrati erano di persone che risiedevano nel villaggio Delabela, centro della zona, o di persone residenti in località rurali vicine, che cercavano assistenza medica in quel centro.

Pertanto, se tenessimo conto che in quell'epoca non c'era un servizio di controllo degli ammalati mediante visite regolari a domicilio, dovremmo concludere che i casi registrati in quel periodo rappresentano appena una frazione certamente inferiore al 50%, degli ammalati realmente esistenti in quella zona.

Durante il periodo dal 1955 all'agosto del 1956 non si fecero prelievi di sangue nel villaggio, ma già nel settembre di quest'ultimo anno, si stabilì un servizio regolare di rilievo dei casi di malaria.

Tre uomini iniziarono visite a domicilio regolari in tutte le case della zona, a intervalli di una o due settimane, prelevando campioni di sangue di tutte le persone trovate con febbre o che avevano avuto febbre nell'intervallo tra una visita e l'altra. D'altra parte, si raccoglievano campioni di sangue di tutte le persone che si presentavano al posto medico del villaggio.

In virtù delle norme sopradescritte, si registrarono in settembre, mese che precedette l'inizio della distribuzione del sale corretto alla popolazione, 36 casi

di malaria e nei primi 20 giorni di ottobre, primo mese di distribuzione del sale antimalarico, 9 casi. Nel novembre si verificò un caso isolato in un individuo residente in un accampamento di lavoratori dei campi dove, benchè già esistesse il sale clorochinato si continuava a fare uso di una rimanenza di sale non corretto; si registrò inoltre un caso importato di una donna residente nel villaggio, ma proveniente da un'altra zona malarica. (8 giorni).

Nei nove mesi seguenti, dal dicembre del 1956 all'agosto del 1957, non si scoprì nessun caso di malaria nella zona. In questo periodo, se non fosse stato per l'uso del sale clorochinato, il numero dei casi sarebbe stato assai maggiore

TABELLA 1.

Area dell'Ingegnere Delabela, Stato di Minas Gerais.
Casi di malaria accertati per mese. Media del quinquennio 1950-54 e dal settembre 1956 al luglio 1958. Ultima applicazione di DDT nell'aprile 1956. Uso del sale clorochinato iniziato il 1° ottobre 1956.

MESE	1950-1954			1956			1957			1958		
	I	II	III	I	II	III	I	II	III	I	II	III
Gennaio	—	34	9	—	—	—	1.387	17	—	1.381	8	1 (3)
Febbraio	—	52	18	—	—	—	1.415	48	—	563	28	—
Marzo	—	51	18	—	—	—	1.437	121	—	517	1.393	9 (4)
Aprile	—	47	15	—	—	—	1.388	18	—	608	1.295	7 (5)
Maggio	—	68	26	—	—	—	1.380	17	—	514	19	—
Giugno	—	54	23	—	—	—	1.722	3	—	524	9	1 (6)
Luglio	—	41	23	—	—	—	1.497	3	—	521	26	—
Agosto	—	48	17	—	—	—	1.981	4	—			
Settembre	—	29	11	1.718	253	36	1.236	10	—			
Ottobre	—	45	15	1.585	162	9	1.803	59	2 (2)			
Novembre	—	28	10	1.380	54	1 (1)	1.121	3	—			
Dicembre	—	23	7	1,525	49	—	1.150	15	1 (3)			
Totale	—	508	187	6.208	518	—	17.544	318	3			

I = N° di visite a domicilio alla ricerca di casi di malaria;
II = N° di persone il cui sangue fu esaminato;
III = N° di persone con plasmodi nel sangue.

OSSERVAZIONI. — (1) Usando sale clorochinato. (2) Sospetti di non usare sale clorochinato. (3) Non investigati (4) Quattro in case con sale semplice; tre con sale clorochinato irregolarmente, con bassa concentrazione di clorochina nell'orina, e due in case con sale clorochinato, ma sospetti di non usarlo. (5) Sei lattanti, da tre a undici mesi di età. Un adulto nella cui casa il sale non conteneva clorochina. (6) Lattanti di 3 mesi.

di quello registrato nel periodo corrispondente del quinquennio 1950-54. Anzitutto perchè in quell'epoca non vi era un servizio regolare di accertamento dei casi di malaria a domicilio, il prelievo del sangue essendo fatto solo sulle persone che frequentavano il posto medico per ottenere medicinali, in secondo luogo perchè nel 1950-54 il DDT era regolarmente applicato nelle abitazioni. due volte all'anno dal 1950 al 1592 e tre volte all'anno dal 1953 al 1955, essendo poi sospeso nell'aprile del 1956.

TABELLA 2.

Guaporanga. Stato di Santa Caterina.
Casi di malaria registrati per mese nel periodo 1955-1957.

(*Secondo* RACHEU *e Collaboratori*, 1958).

MESE	1955	1956	1957
Gennaio	—	6	1 (1)
Febbraio	2	5	—
Marzo	8	10	—
Aprile	15	13	—
Maggio	7	4	—
Giugno	1	4	—
Luglio	2	—	—
Agosto	1	5	—
Settembre	2	5	—
Ottobre	7	9	—
Novembre	2	8	1 (2)
Dicembre	12	7	1 (3)
Totale	59	76	3

OSSERVAZIONI. — (1) Lattante di 10 mesi. (2) Lattante di 10 mesi. (3) Bambino di 3 anni.

Dobbiamo notare che la comparsa di un certo numero di casi di malaria a partire dal settembre 1956, fu dovuta principalmente al forte odore di iodoformio che si sviluppava dall'associazione della clorochina al sale iodato, miscela rifiutata dalla popolazione che doveva essere protetta (6).

Si registrarono 23 casi autoctoni dal settembre 1956 al luglio 1958, dei quali 7 ossia il 30,4% sono di lattanti dai 3 agli 11 mesi di età; l'esame degli

altri casi mostra che alcuni ammalati stavano usando sale semplice e altri, apparentemente, usavano sale clorochinato in maniera irregolare.

4.3. *Guaporanca* (Stato di Santa Caterina). — Villaggio di poche case e di scarsa popolazione, in zona di contagio da *A. cruzii cruzii* e *A. bellator,* considerata come un focolaio di malaria in parte refrattario al DDT. La rappresentazione di dati mediante la Tabella II e alcune considerazioni al riguardo serviranno a chiarire i risultati ottenuti in questa zona con l'uso del sale clorochinato.

L'ultima applicazione di DDT fu fatta il 13 marzo del 1956 e l'uso del sale clorochinato fu iniziato il 12 dicembre dello stesso anno.

Durante i dodici mesi del 1957 che seguirono l'inizio dell'uso del sale clorochinato da parte della popolazione, si verificarono 3 casi di malaria, mentre nel periodo corrispondente del 1956 se ne registrarono 76. Dei casi del 1957, tutti da *P. vivax,* due sono di lattanti e il terzo di un bambino di 3 anni che ingeriva poco sale, secondo quanto rivelò l'esame dell'orina che presentava una eliminazione di 0,2mg di clorochina base per 100ml di liquido, mentre l'analisi del sale nella sua casa mostrava che conteneva il 0,27% di bifosfato di clorochina.

4.4. *Isola di San Francesco* (Stato di Santa Caterina). — La malaria è qui trasmessa dall'*A. cruzii cruzii* e dall'*A. bellator.* Il controllo veniva fatto con applicazioni a domicilio annuali di DDT, dal 1949 e l'ultima applicazione si fece nel febbraio del 1957. Nel marzo del 1958 si iniziò l'uso del sale clorochinato nell'isola e i risultati preliminari ottenuti sono presentati nella Tabella 3.

Alcune spiegazioni sono necessarie per meglio apprezzare i dati contenuti in questa Tabella. Per le stesse ragioni presentate relativamente ai problemi dell'area dell'ing. Delabela, possiamo dire che il numero di casi di malaria accertati nel periodo antecedente all'inizio della distribuzione del sale clorochinato rappresentano appena una frazione di quelli che in realtà si verificarono in quel periodo, poichè non esisteva un regolare rilievo dei casi, né erano prelevati campioni di sangue di tutte le persone con febbre in atto o appena scomparsa, mentre la maggioranza dei casi scoperti era di persone che si recavano al posto medico in cerca di medicamenti. Ciò mostra che la diminuzione di campioni di sangue, prelevati a partire dall'aprile del 1958, è un indice di riduzione dell'incidenza della malaria. E' bene notare che il numero dei casi nel 1958, avrebbe dovuto esser maggiore di quello verificatosi negli anni precedenti, giacchè l'ultima applicazione di DDT fu fatta nel febbraio del 1957 e nessuna misura di controllo dei portatori fu presa fino all'impiego del sale clorochinato. Senza alcun dubbio, questa diminuzione dei casi di malaria osservata a partire dal marzo del 1958, fu ottenuta grazie esclusivamente al sale clorochinato.

Tabella 3.

Isola di San Francesco. Stato di Santa Caterina.
Casi di malaria accertati da gennaio a luglio.
Periodo 1955-1958.

MESE	1955			1956			1957			1958		
	I	II	III	I	II	III	I	II	III	I	II	III
Gennaio	—	51	4	—	169	65	—	208	66	—	169	62
Febbraio	—	81	25	—	204	87	—	265	98	—	176	53
Marzo	—	134	32	—	271	77	—	211	32	810	101	13
Aprile	—	95	32	—	174	35	—	212	42	1069	57	3 (1)
Maggio	—	90	22	—	80	10	—	156	35	1050	54	3 (2)
Giugno	—	153	22	—	53	5	—	71	15	2132	33	—
Luglio	—	95	2	—	87	12	—	59	12	1631	61	2 (3)
Totale	—	666	139	—	1028	291	—	1182	300	6792	651	

Inizio della distribuzione del sale clorochinato nel marzo del 1958.

Ultima applicazione di DDT nel febbraio del 1957.

Nel 1957 e nel 1958 tutte le infezioni sono dovute al *P. vivax*.

I = N° di visite a domicilio per l'accertamento di casi di malaria.
II = N° di persone il cui sangue fu esaminato.
III = N° di persone con plasmodio nel sangue.

Osservazioni. — (1) Due che usavano da pochi giorni sale clorochinato e uno che usava sale senza clorochina. (2) Un bambino di 8 anni che da vari mesi si nutriva di creme e frutta. Un bambino di 7 anni residente da due mesi in casa con sale senza clorochina. Un lattante di 12 mesi. (3) Un lattante di 4 mesi e un bambino di 3 anni.

5 Problemi del sale clorochinato.

I principali problemi relativi al sale clorochinato secondo la nostra esperienza in Brasile sono:

5.1. L'alta solubilità del fosfato di clorochina nell'acqua può determinare una perdita immediata del medicamento qualora il sale sia saturo di umidità, in zone dove l'umidità dell'aria è molto elevata (9). Per evitare questo inconveniente, fino a quando non si disponga di una soluzione migliore (altro sale di chinino, altro medicamento o sostanza stabilizzatrice del difosfato di chinino nel sale, ecc.), si impiegheranno sacchi impermeabili di materia plastica, per l'imballaggio del sale dopo la clorochinizzazione.

5.2. Riduzione dell'iodato di potassio dovuta alla clorochina, con sviluppo di iodoformio. (9). Questo fenomeno dà al sale un odore sgradevole, che

non lo fa accettare dai consumatori. Così il metodo del sale clorochinato non deve esser impiegato in zone dove è in uso il sale iodato per la lotta contro il gozzo endemico, essendo da notare che l'addizione di piccole quantità di bisolfato impedisce la trasformazione dello iodato in iodoformio. (9).

Nella Regione Amazzonica, il problema del gozzo endemico è di importanza secondaria, ragion per cui la iodatazione del sale destinato a quella regione sarà fatta solo dopo aver risolto il problema della malaria.

5.3 L'impossibilità di eliminazione della clorochina attraverso il latte che lascia senza protezione, col metodo del sale corretto, i bambini lattanti. (5)

Altri individui, bambini inferiori ai 5 anni, infermi con diete aclorurate ecc., possono eventualmente ingerire dosi insufficienti di clorochina.

6. L'applicazione del metodo nell'Amazzonia Brasiliana.

Il metodo che illustriamo e che si sta diffondendo e perfezionando nel Brasile fin dal 1952, fu scelto dalla Campagna per l'Eradicazione della malaria (CEM) dal nostro Paese, come mezzo di lotta contro la malaria nell'Amazzonia Brasiliana, basandosi sugli studi, sulle esperienze e sui risultati ottenuti. Recentemente, nel giugno dell'anno in corso, sotto la direzione del Ministero della Sanità del Brasile e con l'aiuto dell'Organizzazione Mondiale della Sanità e del Punto IV, fu lanciata ufficialmente in Belém, Stato del Parà, la nostra Campagna per l'Eradicazione della Malaria, nell'Amazzonia, avendo come primo obbiettivo immediato la clorochinizzazione del sale che penetra nella regione.

Le operazioni consistono in:

6.1. Installazione, nella sede della Campagna, di una unità fornitrice di miscela-madre (sale triturato, secco e con elevata concentrazione di clorochina) agli stabilimenti;

6.2. Installazione, in tutti gli stabilimenti che macinano il sale, di una unità preparatrice di sale clorochinato (aggiunta di mistura-madre al sale macinato secondo le proporzioni fissate), mantenuta da personale istruito dalla CEM;

6.3. Controllo su:

6.3.1. L'entrata di tutto il sale nella regione, al fine di clorochinizzarlo;

6.3.2. Il lavoro di preparazione del sale clorochinato, mediante dosaggi dei campioni prelevati negli stabilimenti, capace di assicurare il mantenimento dell'esatto tenore del medicamento nel sale trattato;

6.3.3. La penetrazione del sale corretto nelle diverse aree della regione in questione;

6.3.4 Il consumo del sale suddetto da parte della popolazione, verificando la presenza della clorochina nel sale trovato nei magazzini, nei negozi e negli spacci di sale o nelle abitazioni della regione, mediante la prova semplice dell'iodio, o anche, ove tale misura fosse necessaria, il controllo della presenza di clorochina nell'urina degli abitanti della regione.

6.3.5. Valutazione epidemiologica dei risultati.

Quasi tutto il sale che entra nella regione è importato sotto forma di sale grosso dai grandi magazzini importatori localizzati nella città di Belém e dintorni e nella città di Manáus. La prima fase del piano consiste nella clorochinizzazione di tutto il sale macinato in questi magazzini, mentre in una seconda fase, saranno controllate e clorochinizzate le piccole quantità di sale importate direttamente da alcune altre città della zona.

A tutt'oggi, sono già in funzionamento le unità di clorochinizzazione nelle 10 fabbriche che macinano il prodotto, conosciute nello Stato del Pará e, a dicembre, saranno in funzionamento le unità di clorochinizzazione di quelle di Manáus, Stato dell'Amazzonia, completandosi così la prima fase del programma.

Poichè non è possibile la sostituzione del sale già esistente nella regione, e poichè occorreranno dai 6 ai 12 mesi perchè questo venga esaurito, nella maggior parte delle località, il sale corretto alla distribuzione impiegherà 12 mesi o più per giungere ad essere consumato da tutta la popolazione. Per questa ragione, il programma di controllo della malaria con applicazioni di DDT nelle case, che si svolge nell'Amazzonia da un decennio e che protegge quasi tutte le città e i villaggi della regione, proseguirà sino a tutto il 1960, tempo utile perchè tutta la popolazione che consuma sale riceva questo prodotto clorochinizzato.

Ciascuna unità preparatrice di sale clorochinato negli stabilimenti di macinazione, costituita di tre uomini, prepara normalmente 7.500 kg di miscela in 8 ore di lavoro.

In Belém e in Manáus funzioneranno laboratori chimici destinati al controllo dei campioni di sale prelevati nelle fonti di produzione della miscela, nelle rivendite e nelle abitazioni dell'interno. Oltre il controllo della presenza e del tenore della clorochina nel sale, nell'interno della valle, funzionerà un servizio per la valutazione epidemiologica dei risultati, servizio che consterà di una rete di informatori e di un quadro di ispettori della stessa CEM.

Nonostante la complessità del programma e l'esistenza di vari problemi tuttora privi di soluzione più adeguata, non ci resta alcun dubbio che il metodo del sale clorochinato sia il mezzo più efficace attualmente disponibile per risolvere il problema della malaria della regione Amazzonica.

GENERAL CONSIDERATIONS ON THE PLAN OF CAMPAIGN AGAINST MALARIA IN BRAZILIAN AMAZONIA USING THE CHLOROQUINOSED SALT TECHNIQUE

The eradication of malaria from Brazil with DDT alone or other insecticides, may not be completed because of, above all - 1, the possibility of transmission out doors by certain vector species (*A. bellator* and *A. darlingi*); 2, the existance of dwellings which can not be adequately protected with the insecticides; 3, the nomadism or semi-nomadism of certain groups.

In Amazonia, in addition to these factors, there are special conditions which complicate the problem of malaria eradication, such as the vast extent of the territory, the low population density and the scarcity or lack of means of communication and transport.

The chloroquinosed salt method appeared to be a possible solution to the problem of Amazonia and recent results, which are described in detail in the text, show that with this technique it is possible to bring about a rapid and conspicuous diminution in the incidence of malaria. The experience made in Brazil show that problems connected with the application of the method are: 1, the high solubility of chloroquine phosphate which can lead to a loss of medicament when the salt becomes saturated by the humidity; 2, the reduction of the potassium iodate by the chloroquine, leading to the appearance of iodoform which gives the salt an unpleasant taste: 3, the failure of chloroquine to be passed with milk so that suckling infants are left unprotected.

The Campaign for the Eradication of malaria from Amazonia, was launched in June 1959, based on the chloroquinosed salt method with the primary objective of treating with chloroquine all the salt which was to enter the region.

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MALARIA ERADICATION PROGRAMME IN INDIA

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The paper briefly reviews the malaria control operations undertaken in India prior to 1958, the year in which the programme for the eradication of malaria was launched. The plan of malaria eradication and other details of the programme are discussed. Brief outline of the actual working of the programme and the experience gained has been briefly referred to. The author concludes that, if everything goes according to the schedule, malaria, which had been a serious health problem in India for centuries, would cease to exist by the end of the year 1964-65.

The history of malaria control in many parts of the world including India prior to the use of DDT has been documented by many writers. The purpose of this paper is to record the current developments in malaria control leading to eradication of the disease. In doing so we salute once more the early pioneers like the late Professor Bastianelli who laid the foundations for the present developments in India and elsewhere.

The epoch making discovery of the role of mosquitoes in malaria transmission by Ronald Ross was made in India in the year 1897. No where were the implications of Ross's discovery more appreciated than in Italy. Bastianelli, Bignami, and Grassi (1898) accounted the presence of developmental stages of the parasites in the stomachs of two *maculipennis* mosquitoes fed on a *falciparum* carrier.

The enthusiastic efforts of a number of workers to control malaria by larvicidal measures brought out the fact that the measures were of relatively high cost for rural areas. And malaria is essentially the disease of rural areas.

Following de Meillon's (1936) work in Africa, anti-adult measures using pyrethrum in the houses offered renewed hope to control rural malaria (Covell et. al., 1938; Russell and Knipe, 1939). This method was quite successful when applied against a determined house haunting species like *A. culicifacies* and *A. minimus* (Viswanathan, 1941) but not so useful against *A. fluviatilis* (Rao V., 1949).

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Towards the end of Second World War the discovery of DDT and its use in malaria control offered unprecendented possibilities in the large scale control of rural malaria. Between 1946 and 1953, several states such as Bombay, Mysore, Delhi etc. carried out large scale successful antimalaria programmes with indoor residual spraying with DDT in rural areas. At the close of the year 1952-53, about 30 million people out of an estimated 200 living in malarious areas were being protected from the disease at an annual cost of about 15 million rupees. It was roughly estimated at that time that in India as it is today, about 75 million suffered from malaria every year. Out of about 5.5 million total annual deaths from all causes, about 0.8 million were directly attributed to malaria (JASWANT SINGH, 1953). The chief defect of antimalaria campaigns during these years was that it consisted of a series of small, restricted attempts to control the disease.

The Health Survey and Development Committee of the Government of India recommended as early as 1946 the establishment of a comprehensive nation-wide malaria control organisation under a central authority. It was, however, in 1952 that a plan was formulated as a health development programme of the First Five Year Plan for this country. The Indo-American aid made it possible to launch this the national malaria control programme in 1953.

NATIONAL MALARIA CONTROL PROGRAMME

In April, 1953, the National Malaria Control Programme, designed to protect the entire population of about 200 out of an estimated 360 million people living in the malarious areas was inaugurated. The objective of this control programme was to bring down malaria transmission in the country to a level when it would cease to be a major public health problem. Once this had been attained, an agency was to be maintained by the state health authorities to hold down the malaria transmission at that low level indefinitely. The method of malaria control provided was to intercept transmission of malaria through the application of residual insecticides on the inside surfaces of the dwellings applied once, twice or thrice a year-depending upon the local conditions-using a total dosage of 200 mg. of technical DDT or its equivalent per sq. ft. of wall surface annually.

JASWANT SINGH *et al.*, (1957) observed that three years working of this programme i. e. from the year 1953-54 to 1955-56 produced a reduction of 23.1 million cases in the total incidence of the disease, which is quite an impressive figure. By the end of 1957-58, 193.5 control units (each unit staffed and equipped to protect 1 million people) were functioning and nearly 164.96 million people had been reached to afford protection from infection. The malaria indices such as spleen rate, parasite rate, infant parasite rate had been steadily decreasing during the period and by the end of three years the malaria morbidity had been effectively reduced in areas where it was a serious health problem.

Experience gained

The National Malaria Control Programme as revised, envisaged an operational phase of five years and a maintenance phase for an indefinite period thereafter by reduction of frequency or the dosage of spraying. In the course of five years two important and outstanding lessons were learnt. One was the almost complete and continued absence of individuals with malaria parasites in certain areas in the country under control with DDT over a period of years. The desirability of planned eradication as a definite programme was well known by that time in malaria world (Pampana, 1948, 1952, 1954 and 1955) and had been fully outlined by the World Health Organisation Expert Committee on Malaria (Sixth report, Athens, June 1956).

The other was the problem of vector resistance to insecticides. A review of the occurrance of resistance in anopheline vectors to insecticides has been made by Brown (1958). In India also, one anopheline vector species, *A. stephensi* to DDT (Raj Gopalan *et al.*, 1956) had developed resistance in a particular locality. (A number of non-vector anophelines have also developed resistance (Pal, 1958). The development of resistance by anopheline mosquitoes if it is materialised to a serious extent was likely to undo all the accumulated benefits of past so many years of malaria control.

The urgency for the planning for eradication and the termination of routine spraying of insecticides before any serious manifestation of anopheline resistance occurs was accepted. In April, 1958, India joined the ranks of 75 other countries that are currently engaged in nation-wide compaigns to eradicate malaria.

The plan of eradication

The object of the control programme was to protect the population where malaria is above hypoendemic level (spleen rate above 10 per cent). The eradication programme aims at giving protection to the entire population of the country. The total population of India at present is of the order of about 390 millions, of these 230 million are estimated to be residing in hyper/meso-endemic areas. The population in the areas where malaria is hypoendemic is estimated to be 160 million. Eradication implies total elimination or the reduction of the parasite reservoir in human population to such a negligible level that once it has been achieved, there is no danger of transmission even though the vectors return to pre-spray densities after termination of spraying operations; a state of anophelism *sine* malaria.

A programme, to achieve the objective of malaria eradication, has four phases i) preparatory, ii) attack, iii) consolidation and iv) maintenance phase

(Sixth Report, WHO Expert Committee on Malaria June, 1956). So far as the Indian programme is concerned, the period of operation of the National Malaria Control Programme was regarded as the preparatory phase of eradication programme. This however, was suitably modified for the attack phase. The changes made are i) the number of units in hyper/meso endemic areas are raised from 200 to 230, ii) a provision for raising 160 hypo-endemic units to extend the scope of eradication to the entire country, iii) increase in the supply of insecticides, equipment and sufficient man-power to effect total coverage, iv) categorisation of the endemic units into (a) a plain area unit and (b) hilly or difficult area units. The plain area units get a 50 per cent more spraying staff as compared with the staff provided under control programme and the hilly or difficult areas get a 100 per cent more spraying staff, v) additonal staff at different levels to strengthen the supervision.

The plan of the six years eradication programme as envisaged is briefly summarised as follows:

Attack phase (1958-59 to 1960-61).

During this phase the total interruption of transmission over a period of 3 years is to be achieved by spraying residual insecticides. A 100 per cent coverage of the surfaces is aimed at, every human habitation and cattle-shed where mosquitoes can rest being sprayed. During each of these 3 years, every roofed structure in the endemic area is to be sprayed at least twive a year depending upon the transmission season. The number of rounds of insecticidal spray may be more than two in selected areas where it is necessary. In the second year of the attack phase, the spraying operations are extended to cover the remaining 160 million living in hypo-endemic areas and carried through in the third year also. In the hypo-endemic areas, only one round of spray of 100 mgs. per sq. ft. of DDT technical per year is to be given. The assessment of results are made in the attack phase by determining routinely epidemiological and entomological indices. These are to serve to find out the defects which can be remedied as the campaign progresses (1) to rectify and (2) to determine what remains to be achieved. The edipemiological assessment includes morbidity rate, spleen and parasite rates in children, and infant parasite rate. The entomological assessment includes determination of vector density, and survival rate. Constant vigilance is to be kept to detect the cases of physiological resistance by carrying out the routine susceptibility tests and behavouristic resistance by making indoor and autdoor collections of vector species etc. The above indices are likely to give information regarding the degree of interruption of transmission achieved, which is the objective of the attack phase.

Consolidation phase (1960-61 to 1963-64).

The consolidation phase in the eradication programme in India is scheduled to begin in the last year of attack phase i. e in the year 1960-61. The attack phase is to bediscontinued from the following year (1961-62) if during the year of concurrent spraying and surveillance operations, it is found that the criteria for the interruption of spraying are satisfied. The criteria accepted as a prerequisite for the withdrawal of spraying are (a) child spleen index of 5 per cent or less for two consecutive years; (b) child parasite index of 1 per cent or less (c) infant parasite index of 0 per cent for two consecutive years and (d) supplemented by evidence of absence of fresh cases by indigenous transmission by surveillance procedures during the year prior to the contemplated termination of spraying. The consolidation phase is, however, to be continued for a minimum period of 3 years i. e. from 1961-62 to 1963-64 after the interruption of spraying. The criteria for declaring that malaria is eradicated is the complete absence of any new indigenous cases during the period of 3 years of surveillance in the absence of any insecticidal or mass chemotherapeutic measures.

Surveillance procedures:

Surveillance involves:

(a) prompt detection and confirmation by microscopic examination of malaria cases, if any, and their treatment to ensure the prevention of any chance of their giving rise to secondary cases;

(b) the detection of residual foci of malaria infection, institution of epidemiological investigations and initiation of such measures as may be considered necessary for effective elimination of such foci.

The data made available by such surveillance over a period of 3 years are necessary to subtantiate the claim of having established eradication. Surveillance consists of two distinct activities (1) detection of malaria cases and (2) epidemiological investigations and taking appropriate measures as called for. While the detection of malaria cases can be carried out either by: (a) active surveillance and, or (b) passive surveillance, the epidemiological investigations etc. can only be carried out by a trained specialist staff forming the epidemiological surveillance unit.

Surveillance organisation:

Active surveillance:

It is essential that the entire population during the consolidation phase should be visited regularly at periodic intervals in order to promptly detect and treat any parasite carrier present in the area. A reasonably satisfactory method

to ensure this end is to organise active surveillance by house to house visits. Since it will not be practical to examine the blood of every individual in every house during such visits and since it is reasonable to expect that a parasite positive individual will manifest fever at some one or other of fortnightly visits during the season, the sample is restricted to all individuals having fever at the time of visit or those who had fever in between two visits. It is estimated that one worker will be able to carry adequate surveillance in a total population of 10,000 living in 2,000 houses visiting each house once in a fortnight. On this basis, for a unit of 1 million people, 100 persons will be required for the purpose of active surveillance.

So during every visit a surveillance worker is to make a record of fever cases present or those who give a history of fever in the interval between two visits. A blood smear is made from all such cases and a dose of antimalarial (4- aminoquinoline) is administered on the spot. The blood smear is sent to the unit office for examination and if this is found to be positive for malaria parasite, the patient is given a course of treatment with a suitable 8-aminoquinoline. In addition an epidemiological investigation of every parasite positive case is made to determine if it is (i) imported, (ii) sporadic, (iii) induced, (iv) introduced or (v) indigenous suggesting a small focus of authochthonous transmission. Necessary measures are to be taken to eliminate such a focus, if required by resuming spraying.

During the last 3 years pilot experiments for surveillance procedures have been initiated in certain parts of Mysore State. The studies are to test the procedures and the organisation in order to be able to establish them under different situations of communications etc.

Maintenance phase.

Once the criteria for eradication have been satisfied, maintenance phase starts which implies a continued vigilance for any unusual prevalence of fever, investigation by the public health staff for any evidence of malaria and take such preventive measures as may be indicated. A reserve stock of 5 tons of DDT per endemic unit is to be held by the States to meet any emergencies. During this phase, the maintenance is to be regarded as the routine activity of the State Public Health Department. Legislation for the notification of cases during these phases will be considered.

Financial implications.

A planned eradication programme means a great expenditure over a limited period of time as against a more modest expenditure recurring annually in perpetuity under a control programme. Therefore, altrough in the beginning an eradication programme appears to be an expensive project compared to a

control programme, there would be considerable saving in expenditure in the long run. The Indian eradication programme is estimated to cost about 530 million rupees over a period of 6 years commencing 1958-59. The programme is financed by the Indian Government with substantial support from bilateral and international agencies like U.S.T.C.M. and W.H.O.

Organisation

There are 14 states forming the Republic of India with a Central Government at Delhi. The responsibility for the public health is vested in States with respect to the health problems of a local nature. But for the control of the diseases like malaria which affects almost the entire country the system of centralised direction and decentralised execution was considered as the most suitable. This system worked quite admirably in case of National Malaria Control Programme and is being continued in the Eradication Programme.

Central organisation.

The Central organisation of the eradication programme is under the Union Ministry of Health - Director General of Health Services through Director, National Malaria Eradication Programme and is located at the Malaria Institute of India, Delhi. Its chief functions are the supervision and co-ordination of the campaign all over the country imparting training and evaluation of results. The Centre is responsible for the supply of material such as insecticides and spraying equipment to the States. Compilation, analysis of results and consolidation of reports is also the function of the Central Organisation.

Regional organisation.

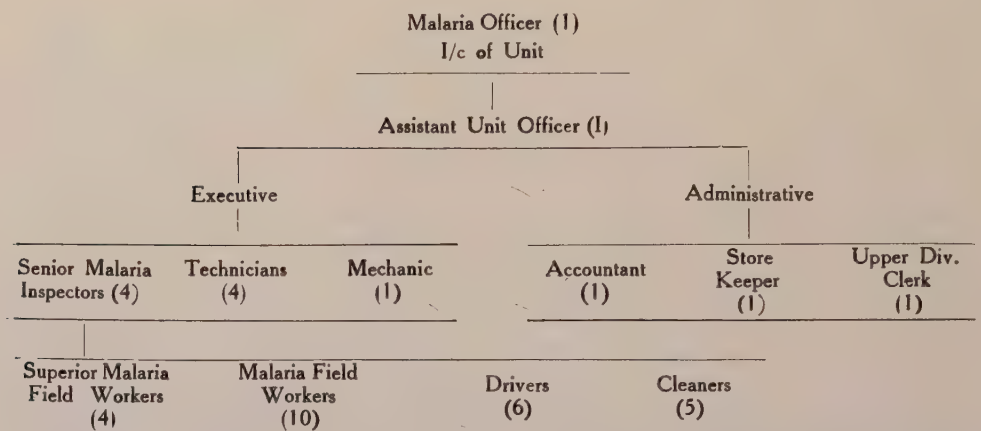
Six regional organisation, each for 2-3 states, have been established in various parts of the country. The regional organisation is under the charge of a Senior Malariologist of the status of a Deputy Director assisted by Malariologists and Entomologists of the ranks of Assistant Director and ancillary staff. The Chief function of the regional organisation is to assist the project in the States and to function as a liaison between the States and the Centre. The regional organisation is also to supervise the work of the units and to suggest any improvements necessary due to the local conditions prevailing for the best execution of the programme. The regional organisations are under the Director, National Malaria Eradication Programme.

State organisation.

Each state is an administrative unit and is responsible for the recruitment and employment of operational staff as also for the actual spraying, assessment

and surveillance operation in each unit of 1 million population established to cover the entire population of the state. Each state has got a Deputy Director/ Assistant Director (Public Health-Malariology) incharge of State Malaria Organisation. The strength of the staff at the states has been strengthened for the better supervison, and for this purpose, zones each covering 5 - 10 units have been formed. Each zone is under an Assistant Director and each unit under a medical afficer trained in malariology. The set up at unit level during the attack phase is shown diagramatically in Table I below:

TABLE 1.



N. B. — Figures in brackts show the number of people working in each category.

Superior field workers and field workers shown in brackets is the permanent staff. During the spraying season the additional number of the workers are employed depending upon the type of the unit. The number and period for which they are employed according the category of the unit are given below:

	Endemic Unit in plains	Endemic Unit in hills	Hypoendamic Unit
Superior field worker for 5 months	32	44	—
Superior field worker for 2½ months	—	—	32
Field workers for 5 months	170	230	—
Field workers for 2½ months	—	—	170

A manual of Malaria Eradication Operation has been prepared in consultation with the State authorities detailing the problem in the country as a whole and in each state, the duties of the different personnel, logistics for spraying and of assessment.

Health education and public relations

The three important activities during the course of a malaria eradication programme viz. total coverage, case finding and complete treatment of cases can be achieved only if there is a willing and understanding participation by the people. To generate such understanding and active co-operation, they will have to be educated about the salient features of this gigantic programme. The nature and purpose of the different measures to be adopted and the benefit expected to accrue to the individuals, communities and the nation as a whole, both socially and economically are being constantly brougth to the notice of the people.

The role of village leaders, school teachers and medical practitioners is extremely important for the implementation of this programme. To secure closer co-operation from the public and promote better understanding for this programme, the public relations activity has been intensified. Special pamphlets, posters and book-lets have been circulated giving the objectives of Malaria Eradication. Cinema slides in English, Hindi and local languages are being widely exhibited. Popular talks on malaria eradication from radio stations of A.I.R. by malariologists have been and will continue to be broadcast from time to time. Special supplements giving details of malaria eradication have been published in daily newspapers and periodicals.

Training of personnel

The proper execution of malaria eradication programme rests with malaria control personnel who must be suitably trained to carry out their duties in an efficient manner. In India, the need and importance of training malaria control personnel was recognised as far back as 1910, when the first training course was held. The training programme had been continually going on since 1925 and was stepped up in the year 1953 when the National Malaria Control Programme was launched under the Malaria Institute of India which has now developed to meet all the local needs in addition to offering training facilities for the countries of the South East Asian region. Besides, the Malaria Institute on India, at Delhi, some of the states in India have also got facilities for local training.

For a programme of malaria eradication of the dimensions of the Indian Programme many trained personnel are needed. The requirements of trained personnel for the 390 units are estimated as follows:

Medical Officers	—	390
Malaria Inspectors	—	3120
Technicians	—	780

As it was not possible to train all the staff in such a short time at the Malaria Institute of India, the facilities were fully utilised for training the medical men and arrangements were set up for training the Malaria Inspectors and technicians in the States and at Regional Headquarters. A considerable number of the Medical Officers and Malaria Inspectors required for the attack phase have been trained. The technicians required for the surveillance phase are being trained. Almost all of the staff required under programme will be trained by the end of 1959-60.

Brief outline of the actual working

During 1958-59, 225.7 units were functioning against 230 units allotted, affording protection to 214 million people. An analysis of the spraying data for the year has revealed that the target of 100 per cent coverage of all shelters twice within the specified time has been reached in only a few of the 230 units covering the country. It was envisaged that the attack phase on the eradication pattern will start in all the endemic areas from the year 1958-59 and all the units would enter the attack and concurrent surveillance phase during the year 1960-61 simultaneously. Actually, during the first year of attack phase, only 50 units could function on the eradication pattern and afford complete protection. The other units could not function according to the plan due to certain administrative difficulties which are apparently inevitable in programmes of this magnitude. Moreover, while formulating the plan it was assumed that in the endemic areas, malaria control units would have functioned for some time and would afford smooth transformation into the malaria eradication units. Thirty units, however, which were allotted in the beginning of the year 1957-58 had not sufficient time to function fully before the launching of Malaria Eradication Programme. In addition to these 30 units, very few of 38 units which were allotted in the year 1956-57, operated in full strength to effectively carry out spraying operations during the year 1956-57. About 25 of them had not finished even their preparatory phase before the eradication programme was launched. Only 50 units out of 230 endemic units are anticipated to be ready for concurrent surveillance during the last year of the attack phase and for the remaining 180 units, a revision of the logistics for the attack and surveillance phases has become necessary.

Hypo-endemic units: 160 hypo-endemic units are to start functioning during the current year and it is expected that these will be ready to go into surveillance during the year 1960-61.

In the preparation of the programme, emphasis was on the requirements of the remaining 3 years of the 2nd plan period as all planning in country are in relation to plan periods. And ad hoc lump-sum provision only suggested

to indicate that the programme would continue into the third plan period. In preparing detailed plans for the next plan period, a revision of the logistics based on a realistic appreciation of certain problems for which provision would be required have been taken into account. Some of these are now briefly referred to:

Protection of border areas

The necessity for special attention to areas lying adjacent to inter-country borders and the development of synchronised achievement of eradication in the countries concerned is well recognised. There is need for special measures in the border areas within the country till such time as mutually satisfactory arrangements are established on both sides. There is an estimated population of about 20 million living in areas situated on the borders adjoining Pakistan, Nepal, Burma and Goa. According to information at present available except in the case of Burma an eradication programme in the other countries is still under consideration or has been started very recently. It is considered necessary to continue spraying in these for a longer time to avoid possible risks of malaria from adjoining countries.

Further it is estimated that a population of about 5 million live either in remote and difficult areas or are likely to present local technical problems. In such circumstances a total interruption of fresh transmission during the 3 years of attack phase may not be possible. In such areas, the attack phase may have to be extended and measures such as chemotherapy may have to be resorted to, for avoiding any risk of fresh transmission after the interruption of spraying in the neighbouring areas.

The plan, as originally envisaged, provides even in the hypo-endemic areas three years of active surveillance after the interruption of spraying. The data collected so far from the hypo-endemic areas suggest that after two years of active surveillance, it may be replaced by passive surveillance supplemented by mobile squads for active surveillance to be deployed in such areas which require special attention.

Revised programme

A revised tentative plan for eradication has been drawn up phasing withdrawal of spraying and surveillance, making adequate provision for the problems indicated. According to the revised draft plan, spraying with concurrent surveillance is to commence in 205 units out of the 230 andemic units and all the hypo-endemic units (160) in the years 1960-61. In the rest of the 25 endemic units which are situated either in the border or difficult areas, the

spraying is proposed to be continued without surveillance till 1964-65 to avoid the risk of fresh transmission.

In the hyper and meso-endemic areas active surveillance is to continue for 3 years after interruption of spraying. In the hypo-endemic areas it is considered that one year of active surveillance after a year of concurrent spraying and surveillance will be sufficient for most of the hypo-endemic units with some modifications. However, to detect any unforeseen foci of transmission in these areas, it is proposed to establish 20 mobile surveillance units which would covere the whole of the hypo-endemic areas in the remaining two years of the surveillance phase.

Table II briefly gives the sequence of spraying, concurrent spraying with surveillance and surveillance only, as regards to the eradication units in each year of the programme from 1958-59, till the expected completion of the eradication programme in 1965-66 at the end of the Third Five Year Plan in India.

Eradication units in each year of the programme, under a revised plan.

TABLE 2.

YEAR	230 endemic units			160 hypo-endemic units		
	Only spraying units	Spraying surveillance units	Only surveillance units	Spraying	Spraying & surveillance	Surveillance only
1958-59	230	—	—	—	—	—
1959-60	230	—	—	160	—	—
1960-61	25	205	—	—	160	—
1961-62	25	155	50	—	70	90
1962-63	25	105	100	—	—	20+20M =40
1963-64	25	50	155	—	—	20M
1964-65	25	—	155	—	—	—
1965-66	—	25	105	—	—	—

M— Mobile units.

As revised, the Indian Eradication Programme will extend over a period of 8 years commencing from the year 1958-59 and will roughly cost about 1035 million rupees. Assuming these estimates reach 1000 million of actual expenditure and the population of the country is 400 million, it would have cost just 2-8-0 per head of population over a period of 8 years or about 5 annas (6 cents) per head per year, a sum considerably less than the cost of a single course of treatment for malaria.

If everything goes according to the plan, the disease will cease to exist in the whole of the country by 1965-66. It would be evident from the complexities of epidemiological factor, and the population to be protected makes the Indian Programme unique and a most imaginative venture in the domain of modern public health.

IL PROGRAMMA DI ERADICAZIONE DELLA MALARIA IN INDIA

Il lavoro riassume brevemente il Programma di Eradicazione della Malaria in India e traccia la sua storia a partire dal 1953, quando venne varato un programma di controllo.

Il lavoro di cinque anni del Programma Nazionale di Lotta Antimalarica ha prodotto risultati assai notevoli e la morbilità per la malattia è stata effettivamente ridotta. A causa della mutata strategia nella prevenzione della malaria, anche l'India ha promosso nell'aprile del 1958 una Campagna nazionale per l'eradicazione della malaria.

Il Programma Nazionale di Eradicazione della Malaria tende a dare protezione all'intera popolazione del paese, stimata dell'ordine di 390.000.000 di individui, 230.000.000 dei quali risiedono in aree iper- e mesoendemiche, mentre i restanti 160.000.000 vivono in aree ipo-endemiche. Il trattamento totale delle aree iper- e meso-endemiche iniziò dal 1958:59, e fu esteso alle zone ipo-endemiche nel 1959-60. Una attiva sorveglianza sarà iniziata durante l'ultimo anno di irrorazione, e cioè nel 1960-61. L'irrorazione cesserà nell'anno seguente, se saranno soddisfatti i criteri per la sua interruzione. La sorveglianza continuerà per un periodo di tre anni, onde dare sostanza all'affermazione di aver raggiunta l'eradicazione, quindi la speciale procedura per l'eradicazione verrà sospesa. Ai fini del successo di un programma così gigantesco sono stati effettuati i necessari preparativi: per un personale di supervisione adeguato, per il materiale, per l'addestramento del personale, e le relazioni pubbliche e l'educazione sanitaria, ecc.

Si spera che per la fine del 1965-66 la malaria cesserà di esistere in India, dove per secoli è stata un serio problema sanitario.

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DANIEL DRAKE - OUTSTANDING PIONEER IN THE EPIDEMIOLOGY OF MALARIA IN AMERICA

PAUL F. RUSSELL (*)

This paper briefly outlines the biography and some of the contributions of Daniel Drake (1785-1852), a notable student of malaria ecology and epidemiology and an outstanding pioneer in the development of Public Health in America.

> « While such strong characters as Rush, Hosack, and Bigelow were advancing medicine on our seaboard, there was living and working in the West a man of whom we must think as one of the ablest, and perhaps the most versatile, of the physicians that America produced in the first half of the last century: Daniel Drake, a fine example of that splendid Western type which built up a great empire out of the wilderness in the course of less than fifty years ». MUMFORD (1).

INTRODUCTION

DANIEL DRAKE was born in humble circumstances in New Jersey on October 20, 1785. In his third year he was taken with his family to May's Lick, Mason County, Kentucky, where in a log cabin in the wilderness Daniel grew into his teens in great poverty. At age 15 he was sent by his father to Cincinnati, Ohio, to become a physician. A friend, Doctor WILLIAM GOFORTH, the pioneer of Jennerian vaccination in the West, had agreed to receive him into his home and to teach this rough and uncouth lad the arts of medicine. Daniel could read and write, he had intelligence, good health, and ambition, with a precocious understanding of human nature. But a more unlikely prospect for eventual inclusion in a list of the 25 outstanding pioneers in the development of public health in America (2) would be difficult to imagine.

(*) *This paper was prepared while the author, recently retired, was a staff member of The Rockefeller Fundation.*

Drake studied Latin and Botany, he read the medical works of such leaders as Cullen, Haller, Cheselden, Boerhaave, Van Swieten, and Rush. He polished his manners and widened his outlook and so improved himself that, at the age of 19, he became Goforth's partner. He was keenly aware of his own deficiencies and the need for further study so, after a year's practice with Doctor Goforth, who gave him a diploma, Drake went to Philadelphia to attend its Medical School. There he listened to lectures by Rush, Woodhouse, Wistar, and Physick. It is pertinent in this paper, written in tribute to a great Italian physician, to recall with affection that the school at Philadelphia was, so to speak, the great-grandchild of the University of Padua, founded in 1212. For, all members of the Faculty of Medicine at Philadelphia in Drake's time — Shippen, Morgan, Kuhn, Wistar, and Physick — were Edinburgh graduates; and the five professors who constituted the first Faculty of Medicine at Edinburgh, in 1726, had all studied at Leyden; and the first teachers of medicine at the University of Leyden, when it opened in 1575, were all graduates of Padua (3).

After six months, having spent all of his money, Drake went back to his Kentucky home in May's Lick where he practiced for a year. During this period he encountered an epidemic of what was probably typhoid fever. This interested him greatly and turned his attention for the first time to the epidemiology of disease. His careful epidemiological observations were the subject of his first publication, which also constituted the first paper on this subject published by a physician west of the Allegheny Mountains. As Horine (4) wrote, this paper was « the germ plasm of his exhaustive and monumental treatise on the diseases of the Mississippi Valley », discussed below.

In 1807 Drake settled in Cincinnati and thereafter made it his principal home. He married well in 1807, developed a large practice, and studied constantly, especially climatology, geography, and botany in relation to disease. In 1810 he published a small but significant book entitled, *Notice Concerning Cincinnati* (5); and in 1815 he added a more ambitious volume called, *Natural and Statistical View, or Picture of Cincinnati* (6). These publications, which received considerable acclaim in the East, set the pattern for much of Drake's writing and gave evidence of his already profound interest in epidemiology, his keenness of observation, and his ability not only to describe and marshall the facts but also to draw significant conclusions from them.

Unfortunately, space does not permit biographical details which would make clear how Drake, although a busy and successful practitioner of clinical medicine, found time to become a leading pioneer in epidemiology and medical ecology and also in medical education. As regards the latter subject, the historian Garrison described Drake's essays as « far and away, the most important contributions ever made to the subject in the United States » (7).

Moreover, DRAKE taught at one time or another in six medical schools and had an active part in the founding of three of them (1, 4, 8).

DRAKE died of pneumonia on November 6, 1852, according to GARRISON « the greatest physician of the West, and one of the most picturesque figures in American medicine ». Sir WILLIAM OSLER once said: « In many ways Daniel Drake is the most unique figure in the history of American Medicine... » (4).

DRAKE'S CLASSIC PUBLICATION

DRAKE'S crowning achievement was a classic two-volume treatise on medical geography entitled, *A Systematic Treatise, Historical, Etiological, and Practical, on the Principal Diseases of the Interior Valley of North America, as They Appear in the Caucasian, African, Indian, and Esquimaux Varieties of its Population* », published in Cincinnati and based on thirty years of travel and intensive labor (9). DRAKE began the preface of the first volume, issued in 1850, as follows: « The object proposed in the following work, is to give an account of the causes, symptoms, pathology, and treatment, of the principal diseases of an extensive portion of North America — its Interior Valley ». This volume, consisting of 878 pages, is divided into two « books », the first entitled « General Etiology » and the second, « Febrile Diseases ». The three « Parts » of the first book are « Topographical and Hydrographical Etiology », « Climatic Etiology », and « Physiological and Social Etiology ». The second book includes only one part - « Autumnal Fever ». In 1854, after DRAKE's death, the second volume appeared under the editorship of S. H. and F. G. SMITH. This volume of 985 pages consists of chapters prepared by DRAKE for his « Book Second » *viz.* « Febrile Diseases ». Included is Part I, « Autumnal Fever », reproduced as given in the first volume. Added are « Part II, Yellow Fever », « Part III, Typhous Fevers », « Part IV, Eruptive Fevers », and « Part V, Phlogistic Fevers: The Phlegmasiae » (10).

GARRISON, in commenting on this exhaustive and monumental work, stated: « There was nothing like this book in literature, unless it might be Hippocrates on Airs, Waters, and Places, and even Hippocrates made no attempt to map out or triangulate the geographic locale of disease » (7). DRAKE'S data were gathered firsthand as he traveled on foot, by horseback, in oxcarts, canoes, river-boats, and otherwise, over the vast drainage area of the Mississippi River and its tributaries, extending from Canada to the Gulf of Mexico and from Pittsburgh to the Rocky Mountains.

DRAKE'S VIEWS ON AUTUMNAL FEVER

One of DRAKE'S major conclusions was that autumnal fever constituted the principal disease of the great interior valleys of North America. DRAKE preferred the name *autumnal fever* to any of the synonyms he encountered

in his journeys: bilious, intermittent, congestive, miasmatic, malarial, marsh, malignant, chill-fever, ague, fever and ague, dumb ague, and *the* Fever. As regards the word malaria, DRAKE wrote, « ... I wish it understood, that if I should, at any time, use the word malaria, it is merely to designate the remote cause, *whatever* it may be » (9a). For instance, in discussing irrigation as an « artificial cause » of autumnal fever, he suggested: « If the surplus water were returned to the streams by ditches, there would, perhaps, be but little malaria produced; but it is generally suffered to run into the lower flats, and give origin to permanent ponds and marshes » (9b). As another example, DRAKE attributed the prophylactic effects of closing windows at night to « ... *first,* the exclusion of malaria, or the poison which produces autumnal fever... » (9c). Again, « ... but the soil being porous, there is but little malaria » (9d). And, « ... the hot, humid, and malarious coasts of Georgia, Florida, and Alabama »; (9e). DRAKE sometimes used the terms, « malarious diseases » and « miasmatic diseases » (9f).

DRAKE considered that there were three chief « speculations on the efficient cause of autumnal fever », *viz.* the meteoric, malarial, and vegeto-animalcular hypotheses. He noted that advocates of the meteoric hypothesis denied the existence of a special autumnal fever poison and ascribed the disease to the direct, combined action of a hot, humid, and electrical atmosphere. DRAKE's objections included his observations that autumnal fever seldom appears on vessels cruising in the Gulf of Mexico although the temperature may be 80°F. and the air nearly saturated with vapor. He noted that in many parts of Kentucky and Tennessee, where the surface is dry and ridgy and the streams narrow or tortuous, the Fever occurs although the atmospheric humidity is small (9g).

DRAKE told of a millpond on Paint Creek, Ohio, which was usually drained the first of June so that « the rains of that month, washed away the silt and dead plants, and animals; so that the people of the adjoining village of Washington, suffered but little from the Fever » (9h). One year the draining was delayed until July and there were no rains to wash out the basin. There was an epidemic of autumnal fever, « which prevailed most on the side of the village next the pond ». Today, with our knowledge of the habits of the malaria vector, *Anopheles quadrimaculatus,* which in that part of Ohio would multiply greatly in a millpond in June and July, the explanation of the epidemic is clear. But to DRAKE it was only partly so. He saw in the incident refutation of the meteoric hypothesis, « except so far as certain atmospheric conditions may act as exciting causes ». Here was evidence supporting the existence of some deleterious agent or poison dissolved or suspended in the atmosphere.

DRAKE noted that neither suitable temperature nor air moisture alone or in combination can produce autumnal fever. Therefore, « if that cause be not

some conjunction of the ordinary elements and sensible qualities of the atmosphere, it *must* be a poison, dissolved or suspended in it » (9i).

As to the malarial hypothesis, DRAKE noted that the facts observed « teach us that there is, mingled with the soil or resting upon it, a great amount and endless variety of organic matter, both animal and vegetable, to the decomposition of which, and to the resulting new compounds, the malarialists look for the efficient cause of autumnal fever » (9j). DRAKE concluded that his observed facts « undeniably establish a connection between a certain condition of the surface and autumnal fever; but they do not prove the existence of malaria, or a *gas,* which is the efficient cause of the Fever » (9k). He stated that « the assumed undiscovered gas, called malaria, must be of the same character » as the known gases « and, therefore, at all times and places be productive of the same effects. Now, although autumnal fever is a disease of intrinsic uniformity, it shows modifications which have not been explained by the assignment of modifying causes; and without such causes, its diversities constitute an objection to the existence of a single agent of an unchangeable character ». DRAKE concluded, « On the whole, therefore, I must repeat, that while the conditions under which autumnal fever appears, are sufficiently clear to observation, the existence of a special gaseous agent, resulting from them, remains to be proved » (9l).

So DRAKE concluded that the vegeto-animalcular hypothesis best explained his observations. He stated that he used the term « vegeto-animalcular » to express an hypothesis « which ascribes autumnal fever to living organic forms, too small to be seen with the naked eye; and which may belong either to the vegetable or animal kingdom, or partake of the characters of both » (9m). DRAKE emphasized that both the malarial and the vegeto-animalcular explanations « must stand *as mere hypotheses.* Neither can claim the rank of a theory ». DRAKE was impressed by MITCHELL's Lectures on the Cryptogamous Origin of Malarious and Epidemic Fevers, which he read after his own manuscript had gone to press and to which he referred in a short footnote (9n). MITCHELL (11) stated: « The only theoretic view of malaria to which I incline, is that which refers marsh fevers, and some of the epidemic diseases, to a living organic cause, capable of reproduction by germs, as is alleged of contagious diseases; but unlike the latter in this, that the germs are not reproduced by the organism of the sick, but exteriorly to, and independently of, the human body. In other words, that as the germs of contagious diseases are reproduced *in* the body, the germs productive of malaria and other noncontagious diseases are elaborated and re-elaborated *out of* the body, and independently of its agency. One is the product of *person,* the other of *place* ». MITCHELL preferred a fungal to an animalcular hypothesis, a preference « founded on the vast number, extraordinary variety, minuteness, diffusion and climatic peculiarities of the fungi ».

DRAKE's own careful observations led him to the belief that autumnal fever was due to a specific, organic, widely distributed, and living agent. But he recognized the influence of such contributory factors as temperature. For instance, he concluded, most accurately as we now know, that « a continuance for more than two months of a heat equal to sixty degrees [Fahrenheit], is necessary to the development of the Fever. Hence we can understand, why it prevails more in October than April, although their mean temperatures are nearly the same ». Again, « ... we may, however, assume, that a summer temperature of sixty degrees, is necessary to the production of the Fever; and that it will not prevail as an epidemic, where the temperature of that season falls below sixty-five; finally, that if the other conditions favoring its production are deficient, it will cease before those reductions of temperature have been reached » (9o).

As to the fact that « autumnal fever prevails very unequally in different years; and that, in the same locality, it may, in one autumn, be malignant and epidemic, and in another mild and sporadic », DRAKE explained as follows: « ... for we know, that throughout the visible organic domain, reproduction is by no means uniform. A year of great abundance, may be followed by one unproductive, in the vegetable kingdom; and in the animal, one summer and autumn will be infested by insects far beyond another. It has often happened, that musquitoes have been absent, from the banks of the middle portion of the Ohio river, for a year, and in the next appeared in immense numbers. We have but to suppose insect forms of a parallel size, to live under corresponding laws, and the hypothesis now before us, offers an explanation of sickly and healthy seasons » (9p).

Thus DRAKE, on the basis of his own observations, over wide areas and many years, came near to solving some of the mystery of autumnal fever. He was convinced that it was caused by vegetable or animal living organisms. He wrote: « Now, may it not be, that two distinct species of the same natural order of microscopic beings, may produce autumnal fever? May not one be the cause of the intermittents - the other of remittents? may not both act on the system at the same time? and may we not thus explain diversities, which are inexplicable on the malarial hypothesis? » (9q).

Surprisingly, DRAKE wrote: « I cannot, *a priori*, attach much practical importance to a discovery of the *efficient* cause of autumnal fever; and have devoted several pages to its discussion. from deference to my brethren, much more than from my own conviction, of the value of the discovery to which so many minds are directed. Did we know the particular meteoric condition, the gas, or the organized microscopic species which produces the Fever, we should not probably be able to defend ourselves against it, by any precautions, but those which experience has already established; nor should we be able to destroy the efficient cause, without annihilating the conditions under which

it is generated. These conditions are already well known. The individual exposed to them is liable to an attack - he who keeps away remains exempt » (9r).

Accordingly, DRAKE's prime objective was, not to find the pathogen which he believed to exist, but to investigate the topography of disease, to correlate local climatological and geographical conditions with incidence of disease, for example, of autumnal fever. His observations showed that the latter disease was not distributed equally and that its unequal distribution depended on such factors as character of soil, type of vegetation, variations in altitude, surface water, and air temperature.

To DRAKE, keen observer and logical thinker that he was, it apparently never occurred that « musquitoes » might be carriers of the « germs » of autumnal fever. Such an idea had not been clearly stated by anyone prior to the publication date of DRAKE's book, althoungh just a few years later, in 1854, BEAUPERTHUY made an unequivocal accusation against mosquitoes. Two or three others had, however, made suggestive comments, for instance, NOTT in 1848, and LANCISI as far back as 1717 (12).

Nevertheless, DRAKE's contributions to the epidemiology and ecology of autumnal fever were notable, constituting an achievement that surpassed all other work of its kind in magnitude and significance.

« We must welcome the future, remembering it will be the past; and we must respect the past, remembering that once it was all that was humanly possible ». SANTAYANA (13).

DANIEL DRAKE. UN GRANDE PIONIERE DELL'EPIDEMIOLOGIA DELLA MALARIA IN AMERICA.

DANIEL DRAKE (1785-1852) è stato un grande pioniere nello sviluppo della Sanità Pubblica in America. Egli ha portato notevoli contributi all'educazione medica, insegnando in sei scuole di medicina e partecipando attivamente alla fondazione di tre. Sebbene dedito intensamente e con successo all'esercizio della clinica, DRAKE si interessava molto di epidemiologia. La sua opera maggiore è stata un classico trattato in due volumi sulla distribuzione geografica delle principali malattie nell'Interior Valley del Nord America, basato su attente osservazioni fatte in circa trent'anni di viaggi. DRAKE studiò in particolare le febbri malariche, che erano assai comuni. Egli fu a favore dell'ipotesi etiologica vegeto-animalculare, ritenendo che queste febbri fossero dovute ad un agente vivente specifico, organico, largamente distribuito. Egli riconobbe e descrisse dettagliatamente alcuni fattori accessori come il clima e la topografia, il terreno e le acque superficiali. OSLER descrisse DRAKE come « la più singolare figura, sotto molti punti di vista, nella storia della medicina americana » e GARRISON chiamò la pubblicazione di geografia medica di DRAKE « il contributo di gran lunga più importante che sia mai stato fatto in argomento negli Stati Uniti ».

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DIE FÄRBUNG VON PROTOZOEN IM SCHNITTPRÄPARAT

W. SCHULEMANN (*) und H. WURMBACH (**)

Durch weitere Variation der bekannten Verfahren zur Färbung von Gewebsschnitten mit Giemsalösung wurde eine Methode ausgearbeitet, die Protozoen (Plasmodien, Trypanosomen, Coccidien) im Schnittpräparat in denselben Nuancen wie im Ausstrichpräparat färbt. Saure Mucopolysaccharide, Chondromucoide, Ribonucleinsäure und Desoxyribonucleinsäure werden gut erkennbar angefärbt.

Die Arbeit ist verfasst in Erinnerung an GIUSEPPE BASTIANELLI. In tiefer, aufrichtiger Verehrung denke ich nicht nur des grossen Forschers und der Zusammenarbeit mit ihm, sondern vor allem des wahrhaft edlen Mannes und Freundes, dem ich mich wissenschaftlich und menschlich so eng verbunden fühlen durfte (W. SCHULEMANN).

Seit der Darstellung des Methylenblau im Jahre 1876 durch CARO (1) hat dieser Farbsoff sowohl in der Biologie wie zur Färbung von Geweben und Krankheitserregern eine so vielseitige Anwendung gefunden, dass darüber eine fast unübersehbare Literatur entstanden ist. Zusammenfassungen über die älteren Veröffentlichungen finden sich in BEILSTEIN (2) Handbuch der Organischen Chemie, R. KRAUSE (3) Enzyklopädie der Mikroskopischen Technik und A. HEFFTER (4) Handbuch der Pharmakologie. Ueber die neuere Literatur gibt H. HARMS (5) einen sehr guten Ueberblick in dem «Handbuch der Farbstoffe für die Mikroskopie».

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Nach meiner Emeritierung übernahm die Leitung des Institutes Herr Professor DOMENJOZ. Sehr dankbar bin ich ihm dafür, dass er mir die Möglichkeit gibt, meine wissenschaftlichen Arbeiten im gewohnten Rahmen fortzusetzen. Besonders freue ich mich über die Freundschaft und Harmonie, die uns verbindet. (W. SCHULEMANN).

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Von besonderer Bedeutung für die Färbung von Protozoen wurden 1891 einerseits die Anwendung eines Gemisches von Methylenblau mit Eosin durch D. Romanowski (6), andererseits die Beobachtung von P. G. Unna (7), dass « gealterte » Methylenblaulösungen « metachromatisch » färben. Die weitere Ausarbeitung dieser Methoden durch eine grosse Reihe von Forschern (vgl. dazu Harms l.c. (5)) führte dann zur Schaffung der Giemsa - Lösung, die wie die ähnlichen Lösungen nach May-Grünwald, Wright, Leishman u.s.w. breiteste Anwendung insbesondere in der Parasitologie und Hämatologie fanden.

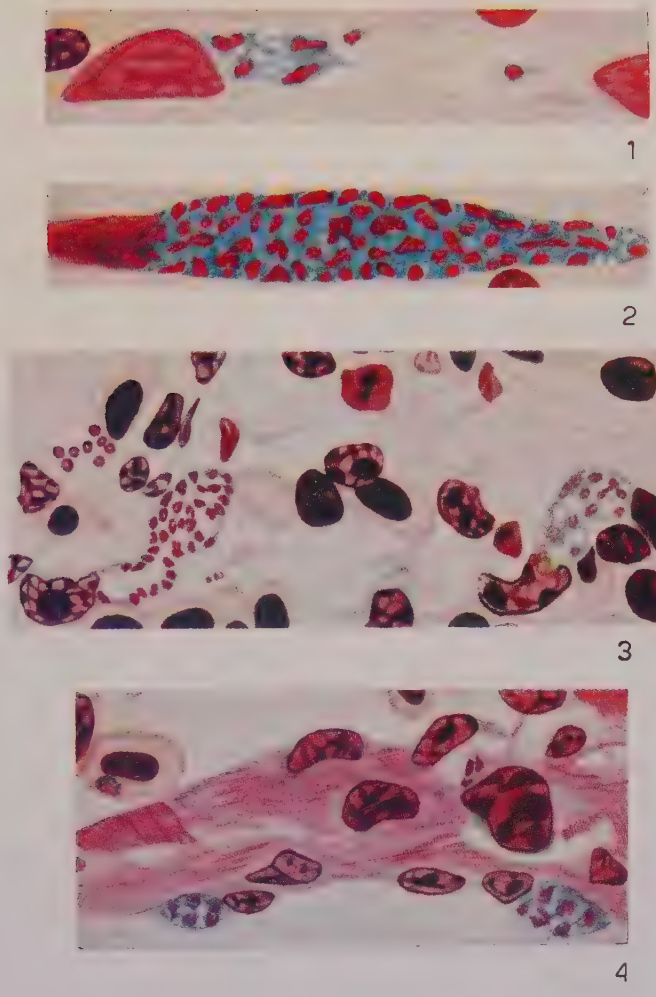
Die färberisch besten Ergebnisse lieferten diese Methoden bei luftgetrockneten und alkoholfixierten Ausstrichpräparaten, während es schwierig blieb, Schnittpräparate gut differenziert zu färben. Vor allem gelang es nicht, Protozoen im Gewebe in derselben Nuancierung wie im Ausstrich darzustellen. A. Maximow (8) versuchte durch Vorfärbung mit Hämatoxylin eine distinktere Färbung des Chromatins in den Parasiten zu erreichen. F. Coulston (9) verbesserte diese Methodik. Wie die Abbildungen z.B. in der Veröffentlichung von C. G. Huff und F. Coulston (10) zeigen, wird das Chromatin der exo-erythrocytären Formen von *P. gallinaceum* zwar sehr gut und scharf, aber doch in der tiefvioletten Nuance des Hämatoxylin, jedoch nicht in dem schönen Purpurrot, wie wir es in den Ausstrichen zu sehen gewohnt sind, gefärbt. Weit bessere Ergebnisse liefert die von W. L. Mc. Namara (11) angegebene Methode der Giemsafärbung, besonders in der von H. E. Shortt und W. Cooper (12) ausgearbeiteten Modifikation.

Bei Arbeiten über die Pathologie und Therapie der Malariainfektion des Vogels erschien es uns notwendig, die Malariaparasiten im Gewebsschnitt genau in derselben Nuancierung anzufärben wie das im Ausstrich möglich ist. Stark alkoholhaltige Fixierungsmittel führen zu starken Zellschrumpfungen. Verwendung von Pikrinsäure oder Chromat-haltigen Gemischen bewirkt — falls sie nicht sorgfältigst ausgewaschen werden — Nuancenverschiebungen des Chromatin.

Zur Fixierung bewährte sich uns das Susa-Gemisch nach Heidenhain (s. Romeis (19)). Seine starke Acidität bewirkt wahrscheinlich eine wenigstens teilweise Hydrolyse der im Chromatin enthaltenen Desoxyribonucleotide, wie das unter Anwendung der Giemsafärbung erstmalig G. Piekarski (13) für die Kernäquivalente in Bakterien, dann aber auch für Trypanosomen (14) durchgeführt hat. Seine Methodik wurde weiterhin erfolgreich ausgebaut von C. F. Robinow (15), M. Piéchaud (16) und anderen mehr. Zweckmässig ist es, nur kleine, dünne Gewebsstücke zu fixieren. Die günstigste Dauer ist unterschiedlich für die einzelnen Organe und Gewebsarten. Ohne mit Wasser auszuwaschen werden die fixierten Stücke über Aethyl- oder Isopropylalkohol unter Zwischenschaltung von Origanum- oder Bergamottoel oder von Benzylbenzoat (als « Weichmacher ») in Paraffin oder Cremolan (Carbowax) eingebettet.

P. CATHEMERIUM

Kanarienvogel



Figur 1 und 2 - Trockenausstriche, übliche Giemsafärbung. Gehirn, Exoerythrocytäre Formen.

Fig. 3 - Schnittpräparat, neue Färbung. Lunge, Exoerythrocytäre Formen in Histiozyten.

Fig. 4 - Schnittpräparat, neue Färbung. Leber, Exoerythrocytäre Formen in Endothelien, Eine Erythrocytäre Form.

Die Schnitte (je nach Notwendigkeit und Ziel von 3 bis zu 10 oder auch 15μ Dicke) werden mit Eiweiss-Glycerin aufgeklebt, das Einbettungsmittel herausgelöst, die Quecksilberverbindungen in üblicher Weise durch Jod-Jodkalilösung und nachfolgende Behandlung mit Natriumthiosulfat entfernt. Nach gründlichem Waschen in dest. Wasser werden die Schnitte für einige Zeit in gepuffertes Wasser (wir verwendeten das Phosphat-Puffergemisch nach Sörensen) eingestellt. Für den Ausfall der Färbungen ist diese Vorbehandlung der Schnitte mit Pufferlösungen und vor allem das Ansetzen der Farblösungen mit Puffergemisch wesentlich. Als günstig für den Nachweis von Parasiten im Gewebe erwies sich uns eine Pufferung auf den pH-Wert von 6,6. (W. Weise (17) und W. L. Mc. Namara (11) halten pH 7,2-7,4 für günstiger). Je mehr der pH-Wert zur alkalischen Seite hin verschoben wird, um so mehr tritt eine graue Allgemeinfärbung ein, die eine scharfe Differenzierung der Parasiten nicht mehr erlaubt. Je mehr umgekehrt die Pufferung zur sauren Seite hin verschoben wird, desto geringer wird die Allgemeinfärbung des Gewebes und es werden mit Methylenblau-Azur schliesslich nur noch diejenigen Substanzen des Gewebes gefärbt, deren isoelektrischer Punkt im stark sauren Bereich liegt wie z.B. Nucleinsäuren und saure Mucopolysaccharide. Ausserdem nimmt dann die Rotfärbung entsprechend zu. Infolgedessen kann die Färbung zum Nachweis der isoelektrischen Punkte der einzelnen Gewebsbestandteile benutzt werden.

Sollen die Gewebsschnitte stärkerer Hydrolyse durch z.B. n/ 1 HCl - Lösung bei + 60° unterworfen oder aber mit Lösungen von Desoxyribonuclease, Ribonuclease oder Hyaluronidase behandelt werden, so muss dies vor dem Einsetzen in die Pufferlösung geschehen.

In den Phosphatpufferlösungen findet bei längerem Stehen leicht starke Bakterienentwicklung statt. Durch Adsorption der Bakterien an den Schnitt kann nach erfolgter Färbung ein Bakteriengehalt des Gewebes vorgetäuscht werden. Um dies sicher zu vermeiden, fanden wir es notwendig, den Pufferlösungen zur Konservierung Thymol zuzusetzen, das den Färbevorgang nicht stört. Ausdrücklich gewarnt sei vor dem Zusatz von Zephirol oder einem ähnlichen Desinfektionsmittel der Reihe der « Invertseifen ». Diese stark oberflächenaktiven Stoffe verhindern eine nachfolgende spezifische Färbung.

Zur Schnittfärbung weniger gut geeignet fanden wir die Verdünnungen der handelsüblichen Giemsalösung in Pufferlösung. Intensiver und besser färbende « Schwebefällungen » erreichten wir durch Mischung der Komponenten.

Selbst bereitet werden zwei Stammlösungen (genaue Vorschrift weiter unten).

Die « Lösung AM » enthält Azur I und Methylenblau,

die « Lösung E » Eosin wasserlöslich, gelblich.

Zur Ausführung der Färbung werden äquivalente Mengen beider Lösungen getrennt in gleichen Mengen Pufferlösung gelöst.

Diese beiden Verdünnungen von Lösung «AM» und «E» werden in genau gleichem Tempo in ein Becherglas zusammengegossen. Es ist überaus wichtig, dass nicht die eine Verdünnung in die andere gegossen wird. Wird Lösung AM in die Lösung E gegossen, so überfärbt das Gemisch nach Rot; wird Lösung E in Lösung AM gegossen, so erfolgt Ueberfärbung nach Blau. Ueber die Grundlagen dieser Vorgänge vergl. die klare Darstellung von R. ZSIGMONDY (18).

Die Schnitte werden bei Zimmertemperatur in Küvetten während 24 Std. gefärbt. Nach dieser Zeit werden sie für weitere 24 Std. nochmals mit neu hergestellter Farbmischung behandelt. Wichtig ist die langdauernde Färbung in sehr stark verdünnter Lösung, um wirklich selektive Färbungen zu bekommen. Nach dem Herausnehmen der Objektträger aus der Farblösung werden diese zunächst in destilliertem Wasser gewaschen und gereinigt (die sorgfältige Wäsche im destillierten Wasser ist unbedingt notwendig, da das in der Farblösung enthaltene gepufferte Wasser — als Salzlösung — nie vollständig entfernt werden kann, dann also Wassertropfen im Schnitt bleiben).

Zur Differenzierung, Entwässerung und Ueberleitung in das Einschlussmittel ist es allgemein üblich, Lösungsmittel zu verwenden, die sowohl in Wasser wie in Kohlenwasserstoffen (Toluol, Xylol u.s.w.) löslich sind.

Methyl- und Aethylalkohol lösen die Farbstoffe zu schnell heraus und geben keine brauchbare Differenzierung. Aehnliches gilt für reines Aceton. Giemsa bremste durch Xylolzusatz die Farbstoff-lösende Wirkung des Aceton ab. Mc. NAMARA (11) und SHORTT u. COOPER (12) setzten zum gleichen Zweck dem Aceton Kolophonium zu.

Uns bewährte sich anfänglich Diacetin (Diacetylglycerin), dann aber gingen wir zu Dioxan über, das auch schon von COULSTON (9) verwendet worden ist.

Zur Differenzierung mit Diacetin ist ein Gemisch von: Diacetin 35 cm^3, Aceton 35 cm^3, Benzol 30 cm^3 geeignet, um die Schnitte dann rasch über Aceton-Xylol-Gemische mit steigendem Xylolgehalt in reines Xylol überzuführen.

Bessere Ergebnisse erzielten wir mit Dioxan. Dabei ist zu beachten, dass in reinem, wasserfreiem Dioxan unsere Farbstoffe praktisch unlöslich sind. Dioxan löst die Farbstoffe nur, wenn es mit Wasser gemischt ist.

Zur Differenzierung sind also nur Mischungen von Dioxan mit Wasser geeignet. Die färberische Erfassung des isoelektrischen Punktes der Gewebsbestandteile ist durch Anwendung einer Mischung von gleichen Teilen Dioxan und Wasser möglich. Anschliessend wird mit reinem Dioxan entwässert.

Sollen Parasiten besonders klar hervorgehoben werden, so empfiehlt sich ein Zusatz kleiner Mengen von Essigsäure zum Gemisch.

Die Dioxan-Mischungen lösen vorwiegend die blauen Farbstoffkomponenten.

Soll eine Differenzierung auch noch der roten Komponenten erfolgen, so kann im Anschluss an die Verwendung der Dioxan-Wasser-Gemische eine Behandlung mit Aceton-Xylol-Gemischen erfolgen.

Zum endgültigen Einschluss der Präparate unter dem Deckglas werden Caedax oder andere Kunststoffe verwendet.

TECHNISCHE ANGABEN

A. *Fixierung*: nach *Susa* [cf ROMEIS (19) *Taschenbuch der Mikroskopischen Technik*].

Kleine Gewebsstücke, nicht dicker als ca 3 mm.

Fixierungsdauer 1-4 Std. Bei Ueberfixierung fällt die Färbung dunkler aus, bei zu schwacher Fixierung zu hell. Die Einhaltung einer konstanten Fixierungsdauer ist daher zur Erzielung konstanter Resultate wesentlich.

B. *Einbettung*: a) in *Cremolan* (Carbowax). Fixierte Gewebsstücke für ca 4-5 Std. in 70% igem Isopropylalkohol. Dann 3 mal gewechseltes Cremolan bei + 54°.

b) in *Paraffin*. Gewebsstücke über 80% igen, dann absoluten Isopropylalkohol in Origanum- oder Bergamottoel oder Benzylbenzoat in

Paraffin vom F +58° (für Schnitte von 4-8μ), oder
» vom F +54° (für Schnitte von 10-15μ).

C. *Ueberführung in Wasser*: Entfernung von Quecksilberverbindungen und evtl. Nachbehandlung mit n/1 HCl, mit Nucleasen etc in üblicher Weise mit Jod-Jodkalium und Natriumthiosulfat. Nach sorgfältigem Auswässern einstellen in

D. Pufferlösung.

Vorbemerkung: Für die Herstellung sämtlicher Lösungen und Verdünnungen ist stets kurz vorher ausgekochtes und eben wieder abgekühltes destilliertes Wasser zu verwenden. Nach Beendigung des Auskochens wird dem noch heissen Wasser Thymol-Lösung zugesetzt.

Herstellung:

3 g Thymol
ad 5 ccm Isopropylalkohol

Auf 1000,0 ccm Wasser werden 0,5 ccm Lösung = 0,3 g Thymol [= 0,03% Thymol] zugesetzt. Umrühren.

Lösung A 9,08 g KH_2PO_4 (nach *Sörensen* [*Merck*])
ad 1000,0 ccm Aqua dest.

Lösung B 11,88 g $Na_2HPO_4, 2H_2O$ (nach *Sörensen* [*Merck*])
ad 1000,0 ccm Aqua dest.

Stammlösung I für pH 7,2

280 ccm Lösung A mischen mit
720 » » B

Daraus *Gebrauchsverdünnung*:

166,0 ccm Stammlösung I
ad 1000,0 ccm Aqua dest.

Stammlösung II für pH 6,6

640 ccm Lösung A
360 ccm » B

Daraus *Gebrauchsverdünnung*:

183,0 ccm Stammlösung II
ad 1000,0 ccm Aqua dest.

E. Färbung.

Farblösung:

1) Lösung AM

1,0 g Azur I (*Merck*) werden in 125 ccm ausgekochtem destill. Wasser suspendiert und 5 Minuten gekocht. Zu dieser noch heissen Azur-Lösung werden 0,5 g Methylenblau med. (*Hoechst*) zugegeben und unter Umschwenken noch ca 5 Min. weiter gekocht. Diese Azur-Methylenblau-Lösung wird noch heiss durch ein angefeuchtetes Faltenfilter filtriert und das Filter mit heissem destilliertem Wasser nachgewaschen bis das Gesamtvolumen der vereinigten Filtrate 250,0 ccm beträgt. Zusatz von 0,2 ccm sub D angegebenen Thymollösung.

2) Lösung E

1,5 g Eosin wasserlöslich, gelblich (*Merck*) werden in 250 ccm heissem dest. Wasser gelöst. Nach dem Abkühlen wird die Lösung durch ein angefeuchtetes Faltenfilter filtriert. Zusatz von Thymol wie sub 1).

Beide Lösungen sind abgefüllt in Flaschen mit Glas- oder Gummistopfen jahrelang haltbar.

Färbung:

Getrennt werden die Lösung AM und E mit gepuffertem Wasser verdünnt unter Verwendung von Erlenmeyer-Kolben.

1) 2,4 ccm Lösung AM ad 1000,0 ccm Pufferlösung
2) 2,8 ccm » E ad 1000,0 ccm »
3) zur Färbung werden Verdünnung 1) und 2) in *genau gleichem Tempo* in ein Becherglas zusammengegossen.

Die Schnitte werden bei Zimmertemperatur in Küvetten während 24 Std. gefärbt. Nach dieser Zeit werden sie für weitere 24 Std. nochmals in neu hergestellter Farbverdünnung weiter gefärbt. Zur Vermeidung des Absetzens der Farbstoffniederschläge in den Schnitten sind die Objektträger schräg geneigt in der Farblösung aufzustellen.

F. *Differenzierung und Entwässerung.*

Lösungen: Zur Differenzierung werden folgende Gemische verwendet:

I	1) Dio I	120,0	ccm	Dioxan	und	80,0	ccm	Aqua	dest.
	2) Dio II	150,0	»	»	»	50,0	»	»	»
	3) Dio III	180,0	»	»	»	20,0	»	»	»
II	1) E I	95,0	»	Aceton	»	5,0	»	»	»
	2) E II	70,0	»	»	»	30,0	»	»	»
	3) E III	30,0	»	»	»	70,0	»	»	»
	4) E IV	5,0	»	»	»	95,0	»	»	»

Zu den 200,0 ccm von Dio I und II werden gegebenenfalls je 2,0 ccm *eines* Gemisches von

1,0 ccm Eisessig mit 35,0 ccm Dioxan zugesetzt, zu Dio III 4,0 ccm.

Der Objektträger mit dem gefärbten Schnitt wird aus der Farblösung herausgenommen und 2 mal in destilliertem Wasser gewaschen. Er wird dann auf Filtrierpapier gelegt und mit einem Stück zusammengefaltetem Filtrierpapier werden Wasser und Farbniederschläge vom Objektträger bis möglichst dicht an die Schnitte heran abgewischt. Der Schnitt selbst muss dabei nass bleiben.

Differenzierung und Entwässerung

	stärker				mittel			schwach	
Dio I	4 x	kurz	eintauchen	oder	1 x	eintauchen	oder	auslassen	
Dio II	3 x	»	»	»	4 x	»	»	3-4 x	tauchen
Dio III	3 x	»	»	»	3 x	»	»	3 x	»

gegebenenfalls weiter

Einbetten über wasserfreies Dioxan.

Soll anschliessend an Dio III noch weiter differenziert werden (cf p. 6):

E I etwa 6 x eintauchen bis die Lösung ohne Schlierenbildung glatt vom Objektträger abläuft,
E II 3 x eintauchen
E III 5 x eintauchen
E IV 5 x eintauchen
Einbetten über Xylol.

Das beste Ergebnis wird erreicht, wenn man den Objektträger in die Gemische immer wieder hineintaucht und herausnimmt bis die Gemische ohne Schlierenbildung glatt von Objekträger ablaufen. Sobald dieser Punkt erreicht ist, bringt man sie in das nächst höhere Gemisch.

Unzweckmässig ist es, die entwässerten Schnitte vor dem Einbetten in Caedax etc stundenlang in Xylol stehen zu lassen. Das kann zu erheblicher Extraction der Rot-Komponente führen.

Ergebnis der Färbung

Die Färbung ist sowohl allgemein für normales und pathologisch verändertes Gewebe wie auch speziell zum Nachweis von Protozoen etc im Schnittpräparat geeignet.

Sie hat sich bisher nicht bewährt zur Färbung von Insekten und Mollusken und müsste für diese weiter modifiziert werden. Das Chromatin der Kerne ist rötlich-violett (metachromatisch) gefärbt, das Cytoplasma je nach Zustand der Zelle hellblau bis rötlich, Erythrocyten sind hellrot, Muskulatur rot und Bindegewebe blassrot bis hellrosa, eosinophile Drüsengranulationen lebhaft hellrot. Die Methode eignet sich also nicht zum spezifischen Nachweis von Bindegewebssubstanzen. Dagegen ist sie ausgezeichnet zum Nachweis von Mucopolysacchariden, z.B. Knorpel oder von Mast - und Schleimzellen. Die Nissl-Schollen der Ganglienzellen sind sehr scharf dargestellt.

Die Darstellung von Malaria-Parasiten im Gewebe lässt sich aus den Abbildungen ersehen. Es ist mit der Methode möglich, selbst kleinste Stadien von Malaria-Parasiten im Gewebe oder von Eimeriden im Darm nachzuweisen. Die Kernfärbung der Parasiten entspricht dabei ihrem Gehalt an Desoxyribonucleinsäure, die wie bei einer histochemischen Reaktion scharf metachromatisch purpur hervortritt. Infolgedessen lässt sich die Methode zum Nachweis von DNS benutzen, wenn ein Vergleichspräparat mit Vorbehandlung mit Desoxyribonuclease hergestellt wird. Das Plasma der jüngeren Parasiten ist gewöhnlich lebhaft blau gefärbt, entsprechend dem reichen Gehalt derselben an Ribonucleinsäure. Anhäufungen von Ribonucleinsäure als Nucleolen im Kern oder in Form von Chromidialkörpern im Plasma werden tief dunkelblau gefärbt.

Ausser für parasitologische Zwecke hat sich die Methode sehr gut bei der Verfolgung des Schicksals der Mucopolysaccharide bei Embryonen

bewährt und ermöglicht vor allem eine sehr klare Darstellung der geringsten Spuren von Chondromucoid in den Knorpelanlagen.

Ausserdem kommt der Methode eine Bedeutung zu für die Verfolgung der Blutbildung im Knochenmark und anderen blutbildenden Geweben.

Die Methode lässt sich auch für Ausstriche anwenden, jedoch müssen diese dann kurz in Susa oder einem anderen sublimathaltigen Fixiermittel *feucht* fixiert werden.

Dank: An der Durchführung der Entwicklungsarbeiten wirkte Fräulein Luisa Kratz verständnisvoll und massgeblich mit. Herrn Günther Schesmer sind wir dankbar für die weitere systematische Durcharbeitung und praktische Auswertung. Herrn Dr. Scholtyseck, der die Methode bei seinen Coccidien-Untersuchungen anwandte, danken wir für die Mitteilung seiner Erfahrungen.

LA COLORAZIONE DEI PROTOZOI NELLE SEZIONI ISTOLOGICHE

I parassiti ed i globuli rossi vengono messi in evidenza mediante una nuova tecnica di colorazione delle sezioni come nei preparati in goccia spessa colorati con il Giemsa. Inoltre i mucopolisaccaridi acidi, ADN e ARN vengono colorati distintamente in violetto tendente al blu, mentre le parti acidofile dei tessuti appaiono rosa. Il metodo è quindi adatto anche per preparati d'insieme e per la dimostrazione delle dette parti tessutali.

Il procedimento consta in una prolungata colorazione in una soluzione tamponata di azzurro-blu di metilene-eosina con successiva differenziazione in una miscela leggermente acidificata di dioxan e acqua; fissazione secondo Heidenhain-Susa; inclusione in caedax.

Per la migliore messa in evidenza dei parassiti la miscela deve avere pH 6,6. Per la preparazione della soluzione azzurro-blu di metilene (AM) si fa bollire g 1 di azzurro I in cc 125 di acqua distillata per 5', poi si aggiunge g 0,5 di blu di metilene medicinale. Dopo altri 5' di ebollizione si filtra e si porta a cc 250. Si fanno infine bollire g 1,5 di eosina idrosolubile in cc 250 di acqua distillata, e si filtra dopo raffreddamento (E). Le soluzioni madri si conservano con aggiunta di timolo. cc 2,4 della soluzione madre AM e cc. 2,8 della soluzione madre E vengono aggiunti ciascuno a cc 100 di soluzione tamponata; ambedue le soluzioni vengono quindi versate in un becker proprio nello stesso momento. Si colora per 24 ore a temperatura ambiente, e poi ancora 24 ore in una soluzione rinnovata del colorante. Dopo lavaggio in acqua distillata, differenziare successivamente in miscele di dioxan e acqua: 120+80; 150+50; 180+20, eventualmente con l'aggiunta di un po' di acido acetico (cc 2 di una miscela di cc 1 di acido acetico glaciale in cc 35 di dioxan). Inclusione in caedax attraverso la miscela acetone-xilolo e xilolo.

STAINING OF PROTOZOAE IN HISTOLOGICAL SECTIONS

The new method of staining tissue sections shows parasites and blood cells in the same way as Giemsa staining of dry smears. Further, it stains acid mucopolysaccharides, D.N.A. and R.N.A. distinctly violet or blue, while acidophil tissue components appear pink. It is thus also suitable for general view specimens and for the demonstration of the above tissue components.

The method consists in long-term staining in a buffered solution of azure — methylene blue — eosin and subsequent differentiation in slightly acidified mixtures of dioxane and water. Fixation is carried out according to Heidenhain-Susa. The sections are mounted in Caedax.

A buffer mixture of pH 6.6 is best suitable for the demonstration of the

parasites. The azure - methylene blue solution (A.M.) is prepared by boiling 1.0 G. azure I in 125 ml distilled water for 5 minutes and then adding 0.5 G. methylene blue of medicinal grade purity. After boiling for another 5 minutes, the solution is filtered and filled up to 250 ml. Separately 1.5 G. water-soluble eosin is boiled in 250 ml distilled water, cooled down and filtered (E). Thymol serves as preservative for these stock solutions. 2.4 ml of stock solution A.M. and 2.8 ml of stock solution E are added to each 100 ml buffer solution, and these two dilutions poured into one beaker at exactly the same speed. Staining in cuvettes at room temperature for 24 hours, and again with fresh stain solution for another 24 hours. After washing in distilled water, each specimen is differentiated successively in dioxane 120 + water 80, dioxane 150 + water 50, dioxane 180 + water 20, if necessary with the addition of a little acetic acid (2 ml of a mixture of 1 ml acetic acid and 35 ml dioxane). Mounting, via acetone-xylol mixtures, and xylol, in Caedax.

LITERATUR

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INFECTION LATENTE ET PRÉMUNITION DANS LE PALUDISME DES PASSEREAUX A *PLASMODIUM RELICTUM*

EDMOND SERGENT (*)

Une étude expérimentale du paludisme des Passereaux à *Plasmodium relictum*, poursuivie sur 6.000 Canaris pendant 60 ans, a montré que l'infection latente métacritique peut atteindre une très longue durée: jusqu'à 8 ans (qui est la durée moyenne de la vie d'un Canari). Cette infection latente confère au Canari la prémunition, résistance acquise solide, qui cesse lorsque guérit l'infection latente.

Pendant 60 ans, nous avons étudié expérimentalement, ETIENNE SERGENT et moi, le Paludisme des Passereaux à *Plasmodium relictum*. Nous avons inoculé près de 6.000 Canaris. Aucun d'eux n'a montré d'immunité innée absolue. Tous ont présenté un accès aigu, phase de lutte ardente entre l'organisme et les Plasmodies, mortelle dans 6 pour cent des cas. Pendant l'accès, les Plasmodies pullulent dans les hématies. Chez les Canaris qui survivent à l'attaque, les symptômes morbides ont disparu, mais l'infection subsiste à l'état torpide. Les Plasmodies végètent, au ralenti, dans les organes internes; elles n'apparaissent dans le sang périphérique que très rarement, pendant peu d'heures et au nombre de quelques unités.

Le phénomène très important, et qui domine l'immunologie du paludisme, est le fait que, tant que dure cette infection latente métacritique, l'organisme, tenu en alerte, résiste à toute attaque d'une autre Plasmodie de la même espèce. Dès que cesse l'infection latente, cette résistance disparaît. L'organisme déparasité redevient sensible à une nouvelle contamination.

Au début de nos recherches, nous avons désigné cette résistance acquise corrélative d'une infection latente sous le nom, qu'avaient employé ALBERT PLEHN et TH. WASIELIEWSKI, d'« immunité relative », et qui la distingue de l'« immunité vraie » absolue, laquelle est consécutive à la guérison à la fois

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TABLEAU 1.

Nombre de cas d'infection latente observés dans les mois qui ont suivi la primo-inoculation.

1er mois	Accès aigu	13e mois	17	25e mois	12	37e mois	2	49e mois	5	61e mois	4	73e mois	3	85e mois	3
2e mois	19	14e mois	8	26e mois	9	38e mois	4	50e mois	2	62e mois	4	74e mois	2	86e mois	
3e mois	13	15e mois	15	27e mois	14	39e mois	11	51e mois	6	63e mois	1	75e mois	1	87e mois	
4e mois	6	16e mois	16	28e mois	11	40e mois	13	52e mois	2	64e mois	5	76e mois		88e mois	
5e mois	8	17e mois	8	29e mois	12	41e mois	8	53e mois	3	65e mois	1	77e mois		89e mois	
6e mois	9	18e mois	10	30e mois	10	42e mois	10	54e mois	3	66e mois	4	78e mois		90e mois	
7e mois	20	19e mois	12	31e mois	15	43e mois	7	55e mois	3	67e mois		79e mois	1	91e mois	
8e mois	16	20e mois	13	32e mois	10	44e mois	3	56e mois	4	68e mois		80e mois		92e mois	
9e mois	13	21e mois	14	33e mois	6	45e mois	5	57e mois	2	69e mois	3	81e mois	2	93e mois	
10e mois	14	22e mois	13	34e mois	9	46e mois	8	58e mois		70e mois	2	82e mois	2	94e mois	
11e mois	16	23e mois	9	35e mois	14	47e mois	3	59e mois	3	71e mois	3	83e mois	1	95e mois	
12e mois	18	24e mois	11	36e mois	5	48e mois	5	60e mois	4	72e mois	1	84e mois	1	96e mois	
1ère année	**152**	**2e année**	**146**	**3e année**	**127**	**4e année**	**79**	**5e année**	**37**	**6e année**	**28**	**7e année**	**13**	**8e année**	**3**

clinique et parasitaire des maladies infectieuses aiguës, telles que la scarlatine, la variole, etc. En 1924, nous avons, pour la clarté du discours, remplacé, avec L. Parrot et A. Donatien, l'appellation d'« immunité relative » par le terme de « prémunition ». Ce terme de « prémunition » s'applique également à d'autres maladies infectieuses chroniques dues à des bactéries, des protozoaires, des champignons et des ultravirus, par exemple la syphilis, la tuberculose, les piroplasmoses, etc.

La présente Note apporte en premier lieu quelques indications numériques sur la durée de l'infection latente métacritique à *P. relictum* chez les Canaris paludéens. L'expérience a porté sur 527 Canaris. L'infection latente a été recherchée: 1) par l'examen microscopique du sang périphérique des Canaris qui, plus ou moins longtemps après la fin de l'accès aigu de première invasion, présentent des symptômes de rechute: « mise en boule », tête sous l'aile, plumage ébouriffé, inactivité et inappétence; 2) chez les Canaris morts naturellement et autopsiés, par l'examen microscopique du sang et des organes internes.

Le Tableau 1 ci-joint montre que chez les 527 Canaris en observation la recherche microscopique des Plasmodies a donné des résultats positifs 585 fois, au cours de 8 années d'observation après la primo-inoculation.

Les Canaris qui ont servi à l'expérience étaient tous âgés de plusieurs mois au moment de leur primo-inoculation. La longévité moyenne de ce Passereau étant de 8 ans, le Tableau 1 où figurent des cas d'infection latente d'une durée de 8 ans montre que l'infection latente peut persister chez des Canaris pendant leur vie entière.

Sur un lot de 110 Canaris primo-inoculés observés depuis 1952, les 182 examens pratiqués après la fin de l'accès aigu ont donné 167 fois des résultats positifs et 15 fois des résultats négatifs. Le Tableau 2 indique la répartition par année des résultats positifs et des négatifs.

Tableau 2.

La recherche de l'infection latente a donné 167 résultats positifs, et 15 négatifs, lors des 182 examens effectués au cours de 8 années sur 110 Canaris.

RESULTATS	ANNÈES							
	I	II	III	IV	V	VI	VII	VIII
Positifs	9	34	41	18	24	25	13	3
Négatifs	4	2	6	1		2		

Sur ce lot de 110 Canaris, les 167 constatations positives provenaient 79 fois de l'examen microscopique du sang périphérique et 88 fois de l'examen microscopique des organes internes pratiqué à l'autopsie.

Voulant apporter également des précisions numériques dans l'étude de la prémunition, nous avons inoculé *P. relictum* une seconde fois à 46 Canaris qui avaient été infectés par une primo-inoculation, et dont l'infection latente avait été vérifiée par 74 examens microscopiques du sang. Ces réinoculations ont été faites aux 46 Canaris à des dates variant de 1 mois à 48 mois après leur primo-inoculation. Le Tableau 3 indique l'ancienneté de leur primo-infection latente au moment où ils ont été réinoculés.

Il ne se produisit aucun accès aigu, ni clinique, ni parasitaire, après leur

Tableau 3.

Ancienneté de l'infection latente due à une primo-inoculation dont étaient porteurs les Canaris au moment de leur réinoculation. Nombre de cas d'infection latente décelés chez eux chaque mois après la primo-inoculation.

1er mois		13e mois	12	25e mois		37e mois	
2e mois		14e mois	3	26e mois		38e mois	1
3e mois		15e mois		27e mois		39e mois	6
4e mois		16e mois		28e mois		40e mois	1
5e mois		17e mois		29e mois	1	41e mois	2
6e mois		18e mois	1	30e mois		42e mois	3
7e mois		19e mois		31e mois		43e mois	2
8e mois		20e mois		32e mois		44e mois	7
9e mois		21e mois		33e mois		45e mois	5
10e mois	1	22e mois		34e mois		46e mois	1
11e mois	8	23e mois		35e mois	1	47e mois	6
12e mois	11	24e mois		36e mois		48e mois	2
1ère année	**20**	**2e année**	**16**	**3e année**	**2**	**4e année**	**36**

TABLEAU 4.

Durée de l'infection latente persistant chez des Canaris réinoculés, après leur seconde inoculation.
Nombre de cas observés chaque mois.

1er mois		13e mois	2	25e mois	5	37e mois	3	49e mois	1	61e mois	
2e mois		14e mois	4	26e mois	1	38e mois	6	50e mois		62e mois	2
3e mois		15e mois	4	27e mois	4	39e mois		51e mois	1	63e mois	
4e mois		16e mois	4	28e mois	1	40e mois	1	52e mois	1	64e mois	
5e mois		17e mois	2	29e mois	4	41e mois	1	53e mois		65e mois	
6e mois		18e mois	3	30e mois	1	42e mois		54e mois		66e mois	
7e mois		19e mois	2	31e mois		43e mois	2	55e mois		67e mois	
8e mois		20e mois	3	32e mois	3	44e mois		56e mois		68e mois	
9e mois		21e mois	1	33e mois	2	45e mois		57e mois		69e mois	
10e mois		22e mois	1	34e mois		46e mois		58e mois	1	70e mois	
11e mois		23e mois	2	35e mois	1	47e mois		59e mois		71e mois	
12e mois		24e mois	3	36e mois	1	48e mois	1	60e mois		72e mois	
1ère année		**2e année**	**31**	**3e année**	**23**	**4e année**	**14**	**5e année**	**4**	**6e année**	**2**

réinoculation, chez les 46 Canaris porteurs d'infection latente. L'examen microscopique a montré souvent chez ces Canaris, après la réinoculation, de très rares parasites: moins de 1 par champ d'objectif à immersion, et qui n'ont apparu dans le sang périphérique que 1 jour ou 2, au maximum 5 jours. C'est ce que nous appelons des « accès de prémunis ». Au contraire, les Canaris, neufs, témoins qui ont été inoculés en même temps avec la même quantité de virus ont tous présenté un accès aigu normal, d'une durée d'environ une semaine, avec grande pullulation des Plasmodies qui ont atteint le nombre de 20 à 30 par champ d'objectif à immersion.

Les 46 Canaris réinoculés, soumis à 74 examens microscopiques du sang, ont présenté une infection latente semblable à celle qu'ils possédaient avant leur réinoculation, et dont la durée a atteint 6 ans, comme le montre le Tableau 4.

Un fait intéressant, que nous avons constaté aussi dans le paludisme des Rongeurs à *Plasmodium berghei,* est la forte virulence potentielle que conservent, pour des animaux neufs, les Plasmodies vivant à l'état d'infection latente, et même à une époque avancée.

En conclusion, le paludisme des Passereaux à *P. relictum,* après l'accès aigu de première invasion, qui a été mortel chez 6 pour cent des Canaris dans nos expériences, présente, chez les individus qui ont survécu, une très longue infection latente, qui peut durer la vie entière du porteur de germes (8 ans).

Pendant ce long espace de temps, l'infection latente confère à l'organisme qu'elle parasite une résistance considérable: la prémunition, contre toute attaque d'une Plasmodie de même espèce. Cette résistance s'est montrée aussi forte après 48 mois qu'après quelques mois de vie latente.

Il y a lieu de noter, à ce propos, qu'à la différence de l'hôte Vertébré, l'hôte Moustique parasité n'acquiert aucune prémunition.

On est tenté, en face de ce phénomène, qui a été constant dans nos expériences, de parler de « mutualisme », c'est-à-dire de l'association de deux êtres animés qui retirent de leur union des bénéfices réciproques, comme celle qui lie les levures de vin aux Drosophiles dans les vignobles. En effet, au cours des longues années où elle a végété à l'état d'infection latente dans les tissus du Vertébré, la Plasmodie a vécu aux dépens de son hôte, mais celui-ci a profité de la protection que lui procurait cette prémunition contre l'assaut d'une autre Plasmodie de même espèce.

INFEZIONE LATENTE E PREMUNIZIONE NELLA MALARIA DEI PASSERACEI DA *PLASMODIUM RELICTUM*

Nessuno di 6.000 canarini iniculati con *P. relictum* nel corso di un assai lungo studio sperimentale della malaria dei Passeracei ha presentato immunità congenita assoluta. Quelli che hanno sopravvissuto all'accesso acuto hanno mostrato uno stato di infezione latente che cinferiva loro la premunizione; il parassita continuando a

vivere a spese dell'ospite ma questo beneficiando di una protezione contro un altro plasmodio della stessa specie.

A mezzo dell'esame microscopico del sangue o degli organi interni è stata ricercata in 527 canarini, da due mesi ad otto anni dopo l'infezione primaria, la durata dell'infezione latente metacritica. Tale ricerca dei plasmodi ha dato risultati positivi 585 volte; e soni stati osservati casi di infezione latente della durata di otto anni (longevità media del canarino).

In un gruppo di 110 canarini osservati dal 1952, da uno ad otto anni dopo l'accesso acuto da *P. relictum*, 182 esami del sangue o degli organi hanno dato risultati positivi 167 volte.

46 canarini la cui infezione latente era stata accertata a mezzo di 74 esami microscopici del sangue, sono stati reinoculati con *P. relictum* dopo da uno a quarantotto mesi dalla prima inoculazione. In seguiti alla reinoculazione sono stati spesso osservati rarissimi parassiti (« accesso dei premuniti »), ma non si è prodotto alcun accesso acuto. Questi canarini reinoculati hanno presentato un'infezione latente simile a quella che avevano prima della reinoculazione, e la cui durata ha raggiunto i sei anni.

LATENT INFECTION AND PREMUNITION IN MALARIA OF THE PASSERINE BIRDS BY *PLASMODIUM RELICTUM*.

Not one of 6.000 canaries inoculated with *P. relictum* during a fairly long experimental study of malaria in the passerine birds showed an absolute congenital immunity. Those which survived the acute attack showed a state of latent infection which conferred on them premunition; the parasite continued to live in a few of the hosts but these benefited by a protection against another plasmodium of the same species.

The duration of the latent metacritical infection was studied in 527 canaries by the microscopical examination of the blood or internal organs between two months and eight years after the primary infection. The search for plasmodia was positive 585 times and cases of latent infection were observed after eight years (the average life of a canary).

In a group of 110 canaries observed in 1952 at one to eight years after an acute attack of *P. relictum*, 182 examinations of blood or internal organs gave 167 positive results.

Forty-seven canaries, whose latent infection had been confirmed by 74 microscopic examinations of blood, were reinoculated with *P. relictum* at from one to forty-eight months after the primary inoculation. Following the reinoculation, a very few parasites were sometimes observed (« a preimunised attack ») but no acute attack was produced. These reinoculated canaries showed a latent infection, similar to that which they had before reinoculation, which has lasted for six years.

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MALARIA ERADICATION AND ITS FUTURE

JASWANT SINGH (*)

A few salient points regarding Malaria Eradication and its future have been discussed in this paper. In 13 countries Malaria Eradication has been achieved and 43 countries have undertaken this task recently. Besides socio-economic benefits a number of trained personnel would be available after the successful conclusion of these programmes to eliminate other important communicable diseases.

GRASSI, BIGNAMI and BASTIANELLI in Italy demonstrated the sporogony cycle of *P. falciparum* in mosquitoes in 1899, soon after the epoch making discovery of Sir RONALD ROSS in Secunderabad (India) in August, 1897. Professor BASTIANELLI, a contemporary of the late Sir RONALD ROSS was, till recently, one of the few survivors of the old vanguard. He lived through the present half century of successive generations of malariologists dedicated to the solution of many of the malaria problems facing the world. BASTIANELLI lived to see malaria eradicated from his beloved country, Italy.

The history of malaria and its control since ancient times has been reviewed by RUSSELL in his series of Heath-Clark lectures in 1953. For our purpose, however, we shall consider only the histroy of malaria prevention since the end of World War II. The discovery of contact insecticides of the hydrocarbon series during the period 1939-1947, on account of its potentialities and its application, completely revolutionised malaria control methods.

Although DDT was synthesised as early as 1874, it was not till 1939 that its insecticidal properties came to be known. Like many things in the past, DDT originally used for some other purpose, in this case as an agricultural pesticide in Switzerland, was used by the malariologists for the control of vector species of masquitoes. By 1943 its potentialities as a powerful insecticide for use against insects of Public Health importance came to be generally recognised. During the period 1943 to 1946 considerable amount of field work was carried out by the Defence organisations and specialised agencies of different countries.

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The new synthetic insecticides not only reduced the cost of malaria control, but also made it possible for the first time to combat the scourge in vast rural areas. When DDT came to be available in large quantities for civilian use additional fillip was given by demonstration teams of the World Health Organisation in different malarious countries. The potent insecticidal action of DDT was largely responsible for the initiation of many National Malaria Control Progammes. Further-more newer synthetic antimalarials have changed the entire rationale of treatment and prevention of malarial infections.

The spectacular successes of large scale malaria control programmes in several countries resulted in a loss of emphasis on basic and fundamental study, i. e. of the bionomics of the vector species on the one hand and the behaviour of the parasite itself in relation to its human host on the other. This situation, however, was not to last long. Soon the lacunae in our knowledge of the behaviour of different malaria vectors in different parts of the world and the need for studying them became apparent. It was perhaps taken for granted that all vector anophelines would be equally susceptible to this insecticide but an analysis of the disappointing results in some parts of the world was summarised by Gabaldon (1953). He classified the anopheline vectors on the basis of their resting and biting habits, and showed that it cannot be taken for granted that all vectors would be equally susceptible to this method of control.

Pal and Sharma (1955) following up Gabaldon's paper reviewed Malaria Control by the use of insecticides from the global stand point. In some areas of Africa and in a few other places, there have been reports of disappointment in its use. The insecticides were reported to exert an excitant effect on certain vectors. Similarly, certain other variations in the behaviour of the adult mosquito vector may tend to lessen the usefulness of residual spraying.

The large scale use of synthetic insecticides for the control of insect-borne diseases led to the development of resistance in the insects in different countries. The earliest record of such a phenomenon was in 1946 with regard to the house-fly in Sweden. Similar observations on resistance to DDT, BHC and organophosphorus compounds have been recorded from Italy, Denmark, North America, Egypt, and other countries (Brown, 1958). Reports regarding the appearance of DDT resistance in *Culex* and *Aëdes* mosquitoes (Bohart and Murray, 1950), King (1950), Deonier et al. (1950) soon became available, sounding a warning note of similar possibilities in anophelines in relation to the malaria control projects. The impact of vector resistance in the field of malaria was first felt in 1951 when *A. sacharovi* of Grece was reported to be resistant to DDT (Livadas and Georgopoulos, 1953). Soon after *A. darlingi* in Southern Brazil and North Argentina was reported to have developed resistance to insecticide (Pinnotti, 1953, and Hess, 1953). Records of insecticide resistance

have since become available regarding a number of important malaria vectors from several countries (QUARTERMAN, 1957).

About the same time as the possibilities of the resistance phenomenon nullifying the potentialities of a weapon which was presumed to be universally effective came to be recognised, there were found large areas in several countries where the general application of insecticides had successfully reduced to negligible proportions, and in some cases even completely eliminated the local incidence of malaria. The necessity for continuing to spray insecticides in the absence of evidence of any local infection was questioned by some of the workers. This formed the basis for the philosophy of eradication of malaria, which was more fully developed later. A total prevention of fresh infections maintained over a period of years should be sufficient to enable the existing reservoirs of infection in the community being eliminated by natural processes, possibly aided by chemotherapy. The withdrawal of measures against the vector may result in their build-up to normal densities, but the disease would disappear resulting in a well recognised situation of anophelism *sine* malaria.

A critical analysis of the experience in countries which had extensive insecticide coverage such as Italy, Venezuela, Greece and Ceylon, side by side with the alarming increase in the reports of resistance to insecticides of vectors of malaria, prompted the World Health Organisation to convene a number of Conferences of malaria workers and urged a revised thinking to tackle this problem from a global stand point. It resulted in the acceptance of not only the feasibility but the urgent necessity for changing over from malaria control to eradication. The resolutions of the Eigth World Health Assembly held in May, 1955 in Mexico city authorised the Director-General of the World Health Organisation to request Governments of malarious countries to give priority to malaria eradication projects in their requests for aid under the United Nations Expanded programme of technical assistance. A Malaria Eradication Special Account (MESA) was also established and the Director-General, World Health Organisation was authorised to obtain financial contributions for malaria eradication from Governmental and private sources.

Table 1 shows a classification of the countries of the world according to the stage of malaria eradication programme as on August, 1, 1957.

From the above tabular statement it is clear that the technical feasibility of malaria eradication stands proved by its achievement in 13 countries. In some of them, viz. Argentina, Italy, Cyprus and the U.S.A. and in a large part of Venezuela, eradication was achieved even before its concept was defined. The birth pangs of launching an eradication programme can be considered to be over in the 43 countries that undertook the task recently.

A malaria eradication programme has a well defined target comparable to that of total war. But unlike in the case of war, in eradication there is a rigid

TABLE 1. (*)

Classification of the countries of the world according of the stage of malaria eradication programme as on August 1, 1957.

Region	Eradication fully or practically achieved.	Eradication being implemented.
African Region	1. Reunion 2. Mauritius	1. Madagascar
American Region	1. Argentina 2. Br. Guiana 3. Fr. Guiana 4. Martinique 5. Puerto Rico 6. U.S.A. 7. Venezuela	1. Bolivia 2. Br. Honduras 3. Br. West Indies 4. Canal Zone 5. Columbia 6. Costa Rica 7. Dominican Republic 8. Ecuador 9. El Salvador 10. Fr. Guadelona 11. Guatemala 12. Haiti 13. Honduras 14. Mexico 15. Nicaragua 16. Panama 17. Paraguay 18. Peru 19. Surinam
Eastern Mediterranean Region	1. Cyprus 2. Gaza Strip	1. Iran 2. Iraq 3. Lebanon 4. Syria
European Region	1. Corsica 2. Italy 3. Netherlands 4. Rumania	1. Albania 2. Bulgaria 3. Byelorussia 4. Greece 5. Turkey 6. Ukraine 7. U.S.S.R. 8. Yugoslavia
S. E. Asia Region	None	1. Afghanistan 2. Burma 3. Ceylon 4. Thailand 5. India (**) 6. Indonesia (**) 7. Nepal (**)
Western Pacific Region	None	1. Cambodia 2. China (Taiwan) 3. Philippines.

(*) Extracted from *Bull. Nat. Soc. Ind. Malaria Mosq. Dis.*, 6, 4, July, 1958.
(**) Eradication Programme started in 1958-59.

time limit to the accomplishment. The achievement of eradication cannot depend on technical competency alone. An all out support in the fields of organisation and administration are essential. In the course of an eradication programme, problems frequently arise in the technical field as well as in the spheres of organisation and administration. Those in the technical field can be solved by concurrent research. Those in the sphere of organisation and administration should not be unsurmountable.

With reasonable optimism one can visualise malaria ceasing to be a continuing problem in the different countries in the foreseeable future. One has to be fully aware that the different countries in the world are likely to reach this stage at different periods of time and in the absence of a biological eradication of the parasite there is need for a continuing awareness of likely local episodes and the means to handle such situation should form a part of the training of all officers, entrusted with the responsibility for the maintenance of public health.

Naturally the question, « After Malaria, what next? » would arise as it is already in the minds of some. While this is understandably a legitimate anxiety on the part of a variety of technicians drawn into this field which by definition is a crash programme limited in time, it is inconceivable that a large body of people with a variety of technical skills may have to go without opportunities for employment, particularly as they would be mostly working in countries with a variety of public health problems. Simultaneously with the increase in tempo of eradication in many parts of the world which also happen to be the so-called underdeveloped areas there is also a visible concerted effort at eliminating other important communicable diseases and setting up a composite public health service. Such a service, to meet the needs of such countries, will have to expand rapidly and there could be no better trained persons than those who have played their part in building up and executing the eradication programmes, which necessitated their reaching every individual house and family in the respective countries.

L'ERADICAZIONE DELLA MALARIA ED IL SUO FUTURO

Il lavoro si riferisce all'evoluzione del concetto di eradicazione della malaria. Spettacolosi progressi nella prevenzione della malaria sono stati fatti dopo la seconda guerra mondiale grazie alla scoperta degli insetticidi di sintesi ed alla loro applicazione in campo pratico per interrompere la trasmissione. Molti Stati hanno dato inizio a programmi di lotta antimalarica su scala nazionale ed hanno ottenuto buoni successi. In qualche caso però l'irrorazione ad effetto residuo delle case non ha dato risultati soddisfacenti, a causa di un differente comportamento delle specie vettrici o, più recentemente, alla comparsa di ceppi dei vettori resistenti agli insetticidi usati. Nonostante ciò l'applicazione generale di insetticidi ha ridotto in diverse nazioni l'incidenza locale della malattia a proporzioni trascurabili, ed in alcuni casi l'ha del tutto eliminata. E' stata di conseguenza posta in discussione la

necessità di proseguire l'irrorazione di insetticidi nella evidente assenza di ogni infezione iocale. La probabilità della comparsa di ceppi resistenti ha causato un cambiamento nella strategia dalla lotta contro la malattia alla sua completa eradicazione.

E' stata ricordata l'importante risoluzione dell'VIII Assemblea dell'O.M.S., ed è stato brevemente riassunto lo status attuale della eradicazione della malaria nel mondo.

Dopo il successo finale dei programmi di eradicazione della malaria un certo quantitativo di personale tecnico specializzato sarà disponibile per altri compiti di sanità pubblica. Questo nucleo di persone sarà probabilmente utile per combattere altre malattie infettive nei diversi paesi.

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INTRODUCTION OF DDT TO ITALY, 1943-1945

FRED L. SOPER (*)

The author tells of testing DDT as a pediculicide and the development of the dusting technique used in the Naples Typhus Outbreak of 1943-1944; of the dramatic rapidity of the break in the epidemic curve, which gave the answer to the threat of post World War II typhus epidemics. DDT was tested as the residual insecticide for reduction of Anopheles at Castel Volturno and Isola Sacra in 1944; a full scale demonstration of complete malaria control was given in 1945 in the Tiber Delta and the Maccarese Plain.

The story of the introduction of DDT to Italy, which occurred late in 1943, and was to bring under rapid control the 1943-44 outbreak of exanthematic typhus in Naples, and the surrounding area, and was to make possible the easy control and eventual eradication of malaria from all Italy, is a fitting subject for a memorial volume to Prof. GIUSEPPE BASTIANELLI, the last to disappear of the early malaria researchers, who tied their names to the discovery of the fundamental elements of the disease.

The Rockefeller Foundation Health Commission (**), mindful of the ravages of typhus among civilian populations during and after military operations throughout the ages, and especially remembering the devastating epidemics of this disease in Eastern Europe and Russia following World War I, made the study of typhus and methods for the control of typhus, in civilian populations, the top priority of its program. Since experience, during and after World War I, had shown that the delousing of civilian populations, by bathing and steam or dryheat treatment of garments, was too slow and cumbersome for effective epidemic control, an early objective of the Commission program was the development of a simple rapid chemical attack on the body louse.

In July, 1943, the author found himself in Algeria, as a member of the

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(*) *Director Emeritus Pan American Sanitary Bureau.*

(**) The Rockfeller Fundation Health Commission was constituted as a special semi-autonomous agency of the Rockefeller Foundation in July 1940 for work in war ravaged areas.

Foundation Commission Typhus Team, collaborating with the Pasteur Institute of Algiers, under the auspices of the Medical Section of NATOUSA (North African Theater of Operations of the United States Army) in the study of methods for the control of epidemic typhus with louse-powders. Preliminary tests with MYL (***) in Mexico (1) and in Egypt (2) had given such promising results that the Typhus Team had resolved to limit its activities to further tests of pediculicides and methods for their mass application, discounting entirely the possibility of epidemic control with vaccines.

As a result of a fortuitous visit by a member of the Typhus Team to the Orlando, Florida, Laboratory of the United States Department of Agriculture, where DDT was undergoing preliminary testing, the Typhus Team had five pounds of the miracle insecticide, of which incredible tales were already beginning to circulate, months before this chemical became available for field testing through regular channels.

The happy results of early tests of DDT at the Maison Carrée Prison (3) in Algiers during the summer of 1943, cut short the search for new pediculicides; and on August 22nd, the application of louse-powder to the skin and to the inner surface of clothing with pumps, without removal of the garments, gave such striking results, that from this date on, the Typhus Team worked only to increase its experience in the application of louse-powder to various population groups, and to determine the best types of hand-and power-pumps to be used. Thus in a few short weeks, what had seemed for some months to be a difficult problem, viz., the rapid delousing of civilian populations, had become ridiculously easy, by the insufflation of insecticide, in a matter of seconds for each person deloused, without bathing, without removal of the clothing, and without special heavy equipment.

The British Broadcasting Company, late in September, 1943, reported the presence of both Cholera and Typhus in Naples, which had not yet been occupied by the Allied Forces. This report proved later to be only partially true; cholera was not found but typhus had been smouldering for some months.

On September 28 and 29, two days before the Allied Forces entered Naples, the RFHC Typhus Team assured the Headquarters of Allied Military Government that the threat of epidemic typhus need no longer be feared since the answer to this threat was known. At the same time, the Typhus Team offered its services to undertake the early solution of the threat announced in Naples. This offer was tentatively accepted by the Allied Military Government Headquarters in North Africa, but no recognition of the serious nature of the threat nor any request for aid was forthcoming from Italy. In the following weeks, the Typhus Team continued with observations in the Maison Carrée

(***) MYL was a special secret insecticidal formulation, devised by the United States Department of Agricolture, and adopted and used by the Armed Forces prior to the introduction of DDT.

Prison, carried out field delousing operations in villages and rural areas in Algeria, and demonstrated techniques and trained sanitary corpsmen of the United States Army in the prisoner of war camps in Algeria, in Marocco, in Tunis and Sicily.

One of the observers of the dusting demonstration at the prisoner of war barracks in Palermo on November 16 wrote, « The simplicity of the dusting techniques and the throughness and rapidity of individual coverage were revelations to the uninitiated. All were convinced that, if the powder would do only a major part of what its sponsors claimed, a revolutionary delousing measure was at hand. The simplicity and speed of operations were astounding to those who were familiar only with various cumbersome and time consuming measures utilizing methyl-bromide, steam, dry-heat, etc. ».

By the end of November, the number of cases of typhus occuring in Naples, at the very beginning of the winter season, became alarming, and, on December 5, the members of the Typhus Team were assigned to work with the Allied Military Government in Naples. * Two members arrived on December 8 and the dusting campaign was inaugurated on December 15, with the compulsory dusting of some 700 passengers, on the first passenger train to leave Naples after the Allied Occupation on October 1.

All of the very early dusting in Naples was done with the MYL powder from US Army stocks. At some time about the end of the year, it was noted that there was a difference in the powder being used, although the packaging was the same. This change marked the unobtrusive switch from MYL to DDT, made without fanfare, to avoid calling the attention of enemy agents to the fact that something better than MYL had been found. Some four hundred pounds of DDT concentrate were flown in but some days were lost in finding adequate diluent and in getting it prepared for use. Thus the relative amounts of MYL and DDT used in Naples cannot be determined, but since most of the early work was done with MYL, the credit for the striking results of the early weeks of the campaign must go to this insecticide.

With the organization of public delousing stations throughout Naples, the population was dusted in droves, and on January 9, when almost 70,000 persons were dusted in a single day, the demand for insecticide had caught up with the supply. But the back of the epidemic had been broken and, now that DDT alone was being used, advantage was taken of its long lasting residual action, and frequent repeated dustings, which were freely administered in the early weeks, were curtailed.

The demonstration in Naples was, thanks to the fact that control measures

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(*) No attempt will be made in the present note to trace the shifting of overall responsibility for the Control of Typhus in Italy, 1943-1945; the Rockefeller Foundation Typhus Team worked continuosly on the problem from December 8, 1943, until after the end of World War II.

had not been taken early in the autumn, a conclusive one; for the first time in public health history, a well-seeded typhus epidemic, rapidly building up in a crowded civilian population, at the beginning of winter, under wartime conditions, had been brought under control.

Although some scattered cases, of what may be termed the typhus outbreak of Naples and the surrounding area, continued to be found until the first days of May, 1944, the dramatic rapidity of the break in the epidemic curve, convinced all concerned that the answer had been given to the problem of potential typhus epidemics during and following World War II. This was amply confirmed by later developments.

Early in the spring of 1944, several weeks before typhus cases ceased to occur, the members of the Rockefeller Foundation Health Commission were invited by the Allied Commission to organize a Malaria Control Demonstration Unit, to undertake studies on the use of DDT for the control of malaria under wartime conditions in Italy. (At the time, the area facing the Allied Armies had been extensively flooded and mined by the German Army; the customary application of larvicides for malaria control was therefore hazardous, if not impossible).

During 1944, work was done on the problem of airplane equipment for spraying DDT in oil as a larvicide and, at the same time, tests were made of methods of applying DDT to the walls of houses at Castel Volturno (4) and of the resultant residual effect on the density of anopheles in the houses. While these studies were under way, Rome was opened to the Allied Forces. The author arrived in Rome on June 21, not long after the departure of the German troops. On June 22, a visit was made to the Director of the Instituto di Malariologia «E. Marchiafava», Professor Bastianelli, to inform him of recent developments and to request that the services of Dr. G. Casini, of the Institute, who had been working with the members of the Health Commission in Naples, first on typhus control and then on malaria studies, be continued with the Commission study program.

A visit on the same day to the Instituto Superiore di Sanità, led to a discussion with Professor A. Missiroli of the possibility of eradicating *Anopheles labranchiae* from Sardinia. This conversation was the seed from which later grew the ERLAAS (*) undertaking (5).

With headquarters established in the Instituto Superiore di Sanità, the Malaria Control Demonstration Unit, collaborated during the summer of 1944, in an Allied Military Government project in the Tiber Delta area, by spraying, with a five percent solution of DDT in kerosene, the interior walls of buildings occupied by troops in Ostia Lido, and of all houses and farm buildings on the

(*) The Ente Regionale per la Lotta Anti-Anofelica in Sardegna (ERLAAS) was established by government decree on April 12, 1946 as a special agency of the Italian High Commission for Hygiene and Public Health.

Isola Sacra. The striking efficiency of DDT in reducing the numbers of Anopheles mosquitoes in human habitations was once more apparent.

During the latter part of 1944, plans were made for a significant trial of DDT in the control of malaria transmission, when used alone, without other measures, in a known highly malarious zone. This 1945 demonstration, for such it proved to be, was designed to determine the effect of a single heavy application, in the early spring, of DDT to the inner walls of all human habitations and animal shelters. The Tiber Delta and the Maccarese Plain were chosen for the demonstration. The advantages of this site were: the availablity of cumulative data on its high malaria potential for many years; the high wartime epidemicity with control by suppressive treatment, rather than prevention of transmission in the preceding year; the difficulty of infiltration of anopheles from outside the area; the variety of agricultural development; the easy accessibility to Rome; and, most important, the agreement of the civil and military authorities to surrender, to the Malaria Control Unit, all responsibility for the control of malaria in the area during the 1945 season.

Professor CRAMAROSSA, Head Public Health Officer of the Hygiene and Health Department for the Comune of Rome, agreed to suspend all antimalaria activity in the test area, and contributed the services of the employees usually engaged in such work to the program.

The military authorities allotted two tons of DDT for the demonstration, at a time when this miracle chemical was still reserved entirely for military objectives. Finally, belief in the success of the demonstration led Professor MISSIROLI to forego all antimalaria measures at Maccarese, where he had carried on control work for many years and where he had, in 1944, made a careful test of the effect of suppressive treatment (6).

The results of the Tiber Delta-Maccarese demonstration (4) are history and need not be repeated here; suffice it to quote Professor MISSIROLI (7): « non un caso di malaria primitiva si è verificata nel Delta del Tevere, ed Ostia ha conseguito una salubrità che non aveva visto da duemila anni in poi, cioè da quando si ebbe l'invasione della Malaria in Italia ».

Professor MISSIROLI had an opportunity to check an epidemic of malaria in mid-season, at Fondi, the same year, by the spraying of DDT during the second half of June. On the basis of his 1945 observations in the Tiber Delta-Maccarese and Fondi areas, Professor MISSIROLI did not hesitate to abandon soil « bonifica » to the agriculturists and to undertake to rid Italy of Malaria in a five year period.

The organization of the Malaria Control Demonstration Unit in 1944 did not interrupt the continuing attention of the Health Commission members to the typhus problem. Following the end of the Naples outbreak early in May 1944, a careful epidemiological analysis of all available data was made and a

definitive report prepared. (8). Cases reported during the post-Naples period were investigated and louse-powder made available where indicated. As North Italy opened up to the Allied Forces, small stocks of DDT were placed at many strategic points in the hands of the local authorities with full instructions for its use. This measure proved to be of great value during the critical period of some weeks immediately following the end of World War II in Europe, when North Italy suddenly became the main highway for hundreds of thousands of refugees from Germany, Austria, Yugoslavia and other countries of Eastern Europe, en route to points in Italy, in France and other countries of Western Europe.

Alarmed by the stories of exanthematic typhus and other diseases in the concentration camps of Germany, the International Red Cross proposed to establish, with staff and equipment from Switzerland, a *cordon sanitaire* from Lake Gard to the Adriatic Sea, where refugees could be screened for contagious disease, bathed and deloused. The Health Commission members were invited to make a first hand reconnaissance of the situation and report on the need for such a *cordon sanitaire.* A rapid visit to the refugee overnight centers which had been improvised in Northern Italy revealed that most of the refugees had been dusted with DDT at some check point before entering Italy; that a few cases of typhus were coming through and that other contagious disease was at a minimum. The recommendation was made that no *cordon sanitaire* should be established, but on the contrary, full advantage should be taken of the favorable weather conditions to move the refugees as rapidly as possible to their destinations, taking only the precaution of delousing (dusting with DDT) such groups as had not been dusted.

The Naples epidemic had served well as a lesson in typhus control and the training ground for the military medical officers with the Allied Forces in Western Europe, who organized the mass dusting of some eighteen million persons in the weeks following the end of the war. The easy rapid movement of refugees through Italy, without undue expense, without the inevitable increase of various contagious diseases in crowded refugee camps, was but a small part of the contribution DDT made to alleviation of the post-war confusion in Europe.

This is part of the story of how DDT came to Italy, where the first dramatic demonstration was made of the practical application of this wonder chemical, which was to free the populations of war-devastated regions from all threats of typhus, during the post-World War years, and was to lead, in a few years, to the disappearance of malaria from Italy, and, in 1955, to the campaign for the world eradication of malaria.

L'INTRODUZIONE DEL DDT IN ITALIA (1943-1945)

Viene fatta la storia delle circostanze in cui il gruppo per lo studio del tifo esantematico della Commissione Sanitaria della Fondazione Rockefeller studiò il DDT quale insetticida contro i pidocchi in Algeria, nel 1943, e sviluppò la tecnica della sua applicazione per polverizzazione che dette risultati così spettacolari nel controllo del tifo esantematico a Napoli e dintorni nel 1943-1944. Napoli si dimostrò una istruttiva base per la preparazione alla situazione di emergenza esistente nei campi di concentramento alla fine della seconda guerra mondiale.

Non appena la situazione del tifo fu ben controllata, fu chiesto ai membri della Fondazione di saggiare la possibilità dell'uso del DDT per il controllo della malaria in Italia. I risultati del suo uso come larvicida furono promettenti, ma vennero rapidamente posti in ombra da quelli sull'uso come insetticida ad azione residua, a Castel Volturno ed all'Isola Sacra, del 1944. La dimostrazione del 1945 della soppressione della trasmissione della malaria mediante l'irrorazione delle pareti interne di tutte le case del delta del Tevere e della pianura di Maccarese, in assenza di ogni altra misura di lotta antimalarica, fu definitiva facendo presagire, come in effetti avvenne, la possibilità dell'eradicazione della malaria dall'Italia entro pochi anni. L'esperienza fatta con il DDT durante l'episodio di tifo esantematico di Napoli, giustificò infine la decisione di opporsi alla creazione di un cordone sanitario, per rallentare il movimento dei profughi della seconda guerra mondiale attraverso l'Italia settentrionale.

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PALUDISME ET PERSPECTIVES DE LA LUTTE ANTIPALUDIQUE DANS LA BASSE GUINÉE (*)

C. Toumanoff (**)

Cette étude concerne le paludisme et l'anophélisme dans la Basse Guinée: ville de Conakry, presqu'île de Kaloum et région de l'estuaire du Rio Nunez.

Les espèces anophéliennes y sont recensées et les indices nosologiques qu'on relève sont rapportés. Des indications sont données afin d'y rendre la lutte antipaludique plus efficace, lutte qui doit être dirigée principalement contre *A. gambiae* var. *melas*.

Depuis les mémorables découvertes faites dans le domaine du paludisme et de l'anophélisme par divers auteurs et entre autres par le Prof. Bastianelli dont nous honorons ici la mémoire, la pratique malariologique courante a fait des progrès considérables.

En se basant sur les faits primordiaux concernant le rôle joué par les moustiques dans la transmission de l'infection palustre, faits établis par Ronald Ross, Grassi, Bignami et Bastianelli, les métodes d'enquêtes épidémiologiques et entomologiques sur le terrain ont été codifiées par Sir Christophers aux Indes, par E. Sergent et ses collaborateurs en Afrique du Nord entre autres, et ont permis de reconnaître l'extension de cette maladie dans le monde, les rapports intimes entre l'anophélisme et le paludisme, l'importance de la physiographie des lieux qui influe sur les gîtes anophéliens, la distribution de ces insectes dans les refuges diurnes ou nocturnes etc...

Depuis les grandes découvertes, des connaissances importantes ont été acquises sur le paludisme, dans diverses parties du monde, tout particulièrement en Afrique Occidentale où elles ne cessent d'être approfondies et où les mesures de lutte antianophélienne ont donné généralement des résultats remarquables.

(*) Cette communication est une revue d'ensemble condensée sur le paludisme et l'anophélisme dans la Basse Guinée qui ont déja fait l'objet de publications antérieures et suivie de considérations générales d'ordre pratique en ce qui concerne la lutte antipalustre dans les régions étudiés.

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Sur certains points cepedant les résultats sont moins bons et c'est le cas, par exemple, de la ville de Conakry, en Basse Guinée.

Ce fait a incité le Service de Santé de la France d'Outre Mer à entreprendre deux enquêtes successives en Guinée, en saison sèche de l'année, enquêtes dont nous fûmes chargés, sur la demande du Ministère, par l'Institut Pasteur de Paris, et qui ont eu pour but de définir les faunes anophéliennes locales et le degré de la nosologie palustre sur la presqu'île de Kaloum et dans une autre région limitée de la Basse Guinée (estuarie du Rio Nunez).

Presqu'ile de Kaloum (Conakry)

Physiographie.

C'est une crête élevée par rapport à la mangrove (plus de 100 mètres), allant du mont Kakoulima jusqu'à la mer. A ce point de la presqu'île s'élève la ville de Conakry.

Cette presqu'île est presque entièrement cuirassée de latérite.

Dans l'ensemble, toute la zone littorale est couverte d'une riche végétation, précédée par endroits d'une zone tropicale caractéristique essentiellement compcsée de palmiers à huile.

Ces palmiers abondent près des zones à palétuviers (*Avicennia*) elles mêmes bordées par une graminée *Paspalum vaginatum,* qui, recouverte d'eau, héberge parfois les larves d'*A. gambiae.*

Mais c'est surtout dans des dépressions de terrains d'alluvion, très fertiles, parcourues par des ruisseaux allant à la mer, qu'on trouve des gîtes favorables au développement des anophélinés.

On observe en outre, en contre bas des routes, des sources qui s'épanchent dans les terrains avoisinants et forment des marécages d'eau douce persistants même en saison sèche.

La presqu'île comporte aussi des gîtes permanents créés par l'homme: lacs artificiels, barrages, canaux d'irrigation... C'est dans ces gîtes que les moustiques passent la période défavorable de la saison sèche.

Recherches entomologiques.

L'enquête effectuée dans la presqu'île de Kaloum pendant la saison sèche en 1956 et 1957 a permis de conclure à la prédominance de 2 anophélinés: *A. gambiae* forme type et *A. gambiae* var. *melas.*

A. gambiae, forme type, se maintient en saison sèche dans quelques gîtes larvaires de fortune qui sont certainement à l'origine de l'expansion de cet anophèle pendant la saison des pluies.

Cette forme est très fréquente en effet, en cette saison, dans les gîtes temporaires qu'on trouve sur la presqu'île.

La forme *melas* est également confinée, en saison sèche, dans des gîtes

larvaires semi-permanents, en amont de ruisseaux d'eau saumâtre, à la limite de la remontée des eaux de mer, gîtes dont la formation coïncide généralement avec la période des vives eaux.

Cet anophèle (var. *melas*) qui ne se rencontre qu'exceptionnellement dans les champs à palétuviers soumis au jeu des marées, peut cepedant y être trouvé dans des anfractuosités permettant la création de gîtes temporaires après la marée haute.

Dans certaines circonstances, quand la forêt de palétuviers est bordée de dunes coquillères et sableuses, on peut même observer le développement permanent d'*A. gambiae* var. *melas*.

Les autres espèces anophéliennes décelées en saison sèche dans la presqu'île de Kaloum à l'état larvaire, dans des gîtes de fortune permanents sont: *coustani* Laveran, *A. obscurus* Grünberg, *A.* (*M.*) *Funestus* Giles, *A.* (*M.*) *hargreavesi* Evans, *A.* (*M*) *hanckoki*, *A.* (*M*) *moucheti*, *A. macmahoni* Evans, *A.* (*C*) *squamosus* Theob., *A.* (*M*) *rufipes*, *A* (*M*) *brunnipes* Theob. et *A.* (*M*) *flavicosta*.

Le comportement des *A. gambiae* adultes (forme type et var. *melas*) est très varié. Si ces moutiques restent parfois dans les villages éloignés de la ville et dans les habitations de la proche banlieue de Conakry pendant le jour, ils ne pénètrent dans les maisons urbaines le plus souvent qu'en fin de soirée pour se gorger et en sortent aussitôt sans se poser sur les murs.

Parmi les espèces anophéliennes signalées, seuls *A. coustani* et *A. obscurus* ont été capturé à l'état d'adultes dans les habitations les autres n'y ont jamais été décelés soit parce qu'elles sont exophiles soit parce qu'étrangères à la région elles s'y introduisent pour pondre, mais ne s'adaptent pas à l'existence sur le terrain aride et en grande partie dégagé de la péninsule.

L'étude des repas sanguins de *A. gambiae* et *A. gambiae* var. *melas* a fait la preuve de leur forte anthropophilie, la plupart des femelles étant gorgées de sang humain.

A cette anthropophilie correspond un indice maxillaire très faible et un haut degré d'infection sporozoïtique, décelé dans 3 villages où furent trouvées surtout les larves d'*A. gambiae* var. *melas*.

Recherches épidémiologiques.

Dans la ville de Conakry, le paludisme se manifeste de façon modérée pendant la saison sèche et le degré d'endémie palustre est plus faible que lors des enquêtes précédentes effectuées en 1938 (Vaucel) et en 1950 (Kopel) où les indices plasmodiques étaient de 58 et 54% contre 11.37% constaté au cours de notre mission.

Le paludisme autochtone existe toujours dans cette ville si l'on en juge d'après l'infection des nourrisons âgés de 1 à 3 mois qui sont nés sur place et n'ont jamais quitté la ville.

Dans la presqu'île de Kaloum le degré d'endémie palustre croit au fur et à mesure qu'on s'éloigne de Conakry, l'indice plasmodique atteignant déjà 30% dans la banlieue de la ville.

A 10 kilomètres environ du centre urbain on observe déjà, en certains endroits, des indices caractérisant une infection très grave (à Manikowondi) ou même une hyperendémicité palustre (à Taouiah, Kakimbo, Ratouma).

Plus à l'intérieur des terres on note tantôt des indices élevés (plus de 50% de porteurs d'hématozoaires), tantôt des indices faibles, le degré d'infection palustre pouvant ainsi être fort différent dans des localités pourtant voisines.

En saison sèche un assez grand nombre de porteurs de gamétocytes ont été décelés dans plusieurs villages de la presqu'île ce qui permet la transmission du paludisme en cette période de l'année, là où les anophèles sont suffisamment nombreux.

Les infections subintrantes ne font d'ailleurs aucun doute et des accès pernicieux ont pu être observés avec pullulation de *Plasmodium falciparum*.

C'est cette forme d'hématozoaire qui domine largement à côté de rares *Pl. vivax* et d'encore plus rares *Pl. malariae*, cette dernière forme étant en régression en Afrique Occidentale.

Chez les enfants l'infection est fréquente entre 1 et 5 ans, moins fréquente au dessus de 5 ans et au-dessous de 1 an. Nous avons mis en évidence un cas de paludisme congénital, sur 43 nouveaux nés examinés sitôt leur naissance. Ceci montre qu'on doit tenir compte de cette éventualité lors des enquêtes sur le paludisme.

Aucun cas d'infection à *Plasmodium ovale* n'a été décelé, cette forme existant cependant dans la zone côtière de la basse Guinée en particulier dans la région du Rio Nunez, plus au nord.

La prèsence de formes indiscutables de *Plasmodium vivax* contredit l'opinion de certains auteurs selon lesquels cette forme n'existerait pas en Afrique Occidentale.

Ces enquêtes ont mis hors de doute l'existence du paludisme autochtone dans la ville de Conakry et le maintien de sa transmission malgré la lutte contre les adultes par traitement mural aux insecticides de contact et la disparition apparente des anophèles.

La présence de ces cas autochtones peut s'expliquer par le comportement spécial d'*A. gambiae*, cette espèce (notamment la var. *melas*) ne stationnant qu'exceptionnellement dans les habitations du centre urbain pendant la saison sèche.

Region du Rio Nunez

Physiographie.

Cette région présente deux sites physiographiques distincts:

1) — les terrains proches de la mer ou de la rivière, « zone littorale », recouverte ou non de palétuviers (*Avicennia* et *Rhizophora*) et de la graminée

1

2

3

4

Fig. 1. — Gîte d'*A. gambiae* var. *melas* formé par des infiltrations d'eau de mer à travers la digue de la voie ferrée qui sépare la ville de la mer; gîte dangereux et facile à éliminer par remblai

Fig. 2. — Remblayage d'une mare pour le récupération du terrain. Assainissement progressif rentable, mais insuffisant

Fig. 3. — Vue, à marée basse d'un terrain à palétuviers, séparé de la mer par une digue qu'on voit au loin. L'installation de cette digue a pratiquement fait disparaître la dangereuse espèce anophélienne d'eau saumâtre; *A. gambiae* var. *melas*, et seuls quelques *Culicinés* y ont été récoltés. Terrain défavorable au développement des anophèles du fait de la pollution et des variations continuelles du niveau assurées par les buses placées sous la digue.

Fig. 4. — Remblai parfait ayant permis l'élimination totale des anophélinés dans un quartier où l'indice plasmodique était de 94% et n'est actuellement que de 15,6%.

du genre *Paspalum,* adaptée à la zône maritime: *P. vaginatum,* envahie pendant les vives eaux par l'eau des marées et où se trouvent des gîtes anophéliens, sont permanents ou temporaires, de moutiques tolérant l'eau saumâtre ou adaptés à elle.

2) une zône éloignée du bord de mer, mais influencée par endroits par les eaux saumâtres.

C'est dans l'ensemble, un terrain surélevé pourvu de gîtes d'eau douce permanentes, rares en saison sèche.

Enquête entomologique.

Cette enquête a été effectuée sur la rive gauche du Rio Nunez et l'emplacement du futur port d'évacuation des bauxites ainsi que dans la partie nord-est de cette région.

Sur la côte, *A. gambiae* var. *melas* a été abondamment trouvé a l'état adulte comme à l'état larvaire.

Quelques rares larves d'*A. gambiae* type ont été récoltées dans les rares gîtes d'eau douce subsistant en saison sèche.

La pullulation de cette forme est donc à craindre au début et à la fin de la saison des pluies.

Dans l'ensemble de la région côtière proprement dite, pendant la saison sèche, la faune anophèlienne est assez pauvre en espèces.

Toutefois, dans les gîtes d'eau douce situés à 2 ou 3 kilomètres de la rive du Rio Nunez, *A. obscurus, A. squamosus* et *A. coustani* furent rencontrés.

Dans la région côtière, les 2 formes d'*A. gambiae* (type et *melas*) ont été récoltées dans les cases africaines, de jour comme de nuit.

Par contre, dans l'unique maison européenne du camp provisoire des bauxites (à Tassibili), *A. gambiae* a été trové attaquant l'homme, uniquement de nuit.

La région nord-est du Rio Nunez, éloignée des marigots, est caractérisée par la présence d'*A. gambiae* forme type (établie par des captures larvaires surtout).

Mais le moustique qui présente une pullulation extraordinaire dans toute la region est *Mansonia uniformis; Culex thalassius* Theob. a été également trouvé dans la région côtière à l'état larvaire et adulte.

Enquête épidémiologique.

Les résultats de cette enquête ne sont valables que pour la saison sèche.

Le degré d'infection palustre, d'après les examens de sang des enfants, correspond dans l'ensemble à celui de l'intérieur de la presqu'île de Kaloum. Il est en effet de 57% contre 53% dans la presqu'île.

Les pourcentages locaux d'infection varient sensiblement ici, notamment chez les enfants.

Il y a une discordance frappante entre le faible pourcentage d'infection des adultes et celui, très élevé des enfants, constatation conforme à la plupart des observations en zône endémique.

Dans la zône proche de l'estuaire du Rio Nunez et dans la région du nord-est, on constate un même degré élevé d'infection palustre et un indice plasmodique comparable.

Cependant l'indice parasitaire, chez les enfants de la zône de Kamsar, est plus élevé que dans la region nord-est (64% contre 51%), alors qu'il est inférieur dans la région de Kamsar si l'on tient compte aussi des adultes.

Le parasite prédominant en saison sèche est le *Plasmodium falciparum* (92%), les autres formes étant *Plasmodium vivax* (5%) et *malariae* (3%) tandis que *Plasmodium ovale* ne fut rencontré que dans un seul cas certain.

Des porteurs de gamétocytes ont été trouvés dans toutes les formes de parasitisme, mais surtout chez les paludéens porteurs de *Plasmodium falciparum* (0 à 16% selon les endroits).

L'indice gamétique fut de 8% dans la région de Kamsar, 4% dans la région nord-est, soit 6% en moyenne.

* * *

Il se dégage de nos observations que le paludisme dans la Basse Guinée se manifeste sous un haut degré en saison sèche de l'année dans cette partie de l'Afrique Occidentale.

Malgré une lutte contre les insectes adultes commencée en 1949 et conduite énergiquement dans les conditions requises le paludisme a persisté dans la ville de Conakry quoique à un degré plus faible que dans la presqu'île de Kaloum.

Les insecticides utilisés à l'époque de notre enquête étaient le DDT à 5% dans le pétrole pour le traitement des habitations, l'exapoudre à 10% de HCH (12-14% isomère γ) et l'hexacridol à 25% de HCH (12-14% isomère γ) pour le saupoudrage des cours, buissons et broussailles; le traitement extérieur des cases était assuré enfin par l'actidrine, produit à base de dieldrin (1 bidon de 3 litres dans 100 litres d'eau (Dr. Simond)).

Les résultats des pulvérisation en ville se sont montrés dans l'ensemble plus que satisfaisants et les moutiques même banaux (*Culex fatigans*) n'incommodèrent que pendant une courte période les habitants du centre urbain.

Le contrôle entomologique n'a permis de capturer au début de la saison des pluies, dans un batiment européen moderne, qu'une seule femelle adulte (*A. gambiae*) sur un mur et environ un mois après traitement de ce batiment par une solution de DDT à 5%.

La persistance du paludisme dans la ville de Conakry est apparamment difficile à comprendre. En effet à l'exception de la capture signalée d'un seul

anophèle les recherches effectuées à de nombreuses reprises dans la ville de Conakry et la proche banlieue par nous mêmes, en saison sèche, et par notre collaborateur M. MARCHAL en saison des pluies, n'ont donné que des résultats négatifs.

Il y a donc une discordance entre le degrè de l'anophélisme (constaté dans les habitations) et le paludisme dans la capitale de la Guinée et cette discordance peut être expliquée par le fait d'une exophilie de la faune résiduelle de *A. gambiae* var. *melas*.

L'étude de l'anophélisme et du paludisme que nous avons faite tant sur la presqu'île de Kaloum que dans l'estuarie du Rio Nunez nous conduit à la conclusion que l'utilisation des insecticides organiques de contact n'y peut aboutir à une éradication complète.

C'est surtout la physiographie variée du terrain qui assure dans cette partie de l'Afrique Occidentale le développement des espèces vectrices du paludisme *A. gambiae* et sa variété *melas*, développement difficile à empêcher aussi par la seule lutte antilarvaire. Quant à l'exophilie des adultes elle rend l'usage des insecticides de contact moins efficace qu'ailleurs.

On trouve en effet sur la presqu'île de Kaloum et dans la région du Rio Nunez de nombreux micro-sites physiographiques.

Dans la première de ces régions nous avons décelé en saison sèche de l'année des gîtes permanents assurant le maintien de *A. gambiae* forme type. Ces gîtes sont constitués soit par des marécages d'eau douce alimentés par les eaux de ruissellement soit par des collections d'eau saumâtre, se formant en amont des ruisseaux et influencés par le marées en période de vives eaux.

L'irrégularité du terrain qui s'observe sur la presqu'île de Kaloum fait qu'en saison des pluies il se forme sur son étendue une multitude de gîtes d'eau douce temporaires qui permettent le développement de *A. gambiae* forme type lorsqu'ils sont influencés par les marées et hébergent également la variété *melas*. C'est ainsi qu'au début et à la fin de la saison des pluies on assiste à l'épanouissement du développement de ces deux formes d'*A. gambiae* qui jouent le rôle le plus important dans la transmission du paludisme comme du reste dans toute la zône côtière de l'Afrique Occidentale.

Ainsi, dans la ville de Conakry, sur les terrains proches de la mer et séparés de celle-ci par les digues où les terrains surélevés naturels les collections d'eau temporaires qui se forment pendant les périodes des « vives eaux » sont parfois propices au développement périodique d'*Anopheles gambiae* var. *melas*.

Nous sommes ainsi dans cette region de l'Afrique Occidentale en présence d'une situation curieuse de « paludisme sans anophèles », paludisme faible mais qui persiste et d'une manière évidente.

On ne saurait l'expliquer que par un comportement biologique spécial, consistant soit en une pénétration très temporaire des anophèles dans les

habitations, soit même au fait que les moustiques se nourrissent au dehors de ces habitations.

Ce comportement qui caractérise la ville de Conakry s'oppose a l'exophilie et à l'entophilie vrais qu'on observe dans certaines localités à l'intérieur de la presqu'île.

Il ne s'agit pas là certainement d'un comportement dû à l'effet des insecticides puisque déjà Le Moal en 1906, puis Vaucel en 1938, lorsque le paludisme se manifestait en ville à un plus haut degré et que la lutte antipaludique n'était que limitée, ont noté une rareté extraordinaire des anophélinés dans cette ville.

Cette absence apparente d'anophèles s'observait du reste au cours de nos missions à la périphérie de la ville où le traitement mural des habitations n'a pas été appliqué.

Du point de vue antipaludique c'est la surveillance des gîtes et leur traitement, de préférence avant leur envahissement par l'eau de mer, qui pourrait constituer une mesure utile afin d'empécher l'apparition dans cette ville des anophélinés dangereux qui, grâce à leur exophilie, peuvent échapper aux mesures imagocides.

On doit dire toutefois, que dans le centre urbain de Conakry et sa proche banlieue ce sont les travaux de remblayage qui s'avèreront les plus efficaces sur le plan antimalarien.

Ce même procédé de remblayage progressif pourra aboutir également à l'assainissement du terrain qui environne le futur port d'évacuation des bauxites, terrain dont l'aspect et la nature des gîtes on été décrits antérieurement ailleurs.

Nos observations sur l'anophélisme dans la Basse Guinée ont permis de révéler une fois de plus la complexité de la lutte antipalustre dans la zône côtière, complexité qui exige non pas une mais toute une série de mesures appropriées en rapport avec la nature du terrain.

Ces mesures se confondront avec les travaux d'urbanisme et les travaux d'assainissement d'ordre général. On doit en somme avoir recours aux méthodes anciennes de lutte anti-malarienne: remblais hydrauliques, drainages, endiguements des zônes à palétuviers, règularisation des cours d'eau, des marigots etc... méthodes trop négligées depuis l'emploi des insecticides organiques de contact, à la longue très dispendieux surtout lorsque les résultats ne sont que partiellement rentables.

C'est dans ce sens du reste que s'était orientée durant les dernières années l'administration française en Guinée, et l'assainissement permanent, mais partiel de la ville de Conakry, concurremment avec l'emploi des insecticides, a eté réalisé grâce à l'effort accompli par les autorités de cet ancien territoire de la France d'Outre Mer.

On ne peut que souhaiter que cet effort soit poursuivi pour le bien être de la population guinéenne.

MALARIA E PROSPETTIVE DELLA LOTTA ANTIMALARICA NELLA BASSA GUINEA

Il lavoro rappresenta un compendio delle inchieste effettuate dall'A. nel corso di due missioni sulla malaria e l'anofelismo nella stagione secca in Guinea, onde riconoscere i focolai permanenti e potenziali degli anofelini in Africa Occidentale, nelle zone litorali della penisola di Kaloum e dell'estuario del Rio Nunez.

Sono riportati i dati sul censimento delle specie anofeliche ed alcune indicazioni sul grado della nosologia malarica.

Dallo studio risulta che durante la stagione secca sono presenti nella penisola di Kaloum e nella regione del Rio Nunez, soprattutto allo stato larvale, parecchie specie anofeliche e che la maggior parte di esse non rappresentano verosimilmente la fauna autoctona fissa. Esse vengono probabilmente introdotte nella penisola di Kaloum dalla Media ed Alta Guinea e non si adattano a questa regione arida in cui il loro sviluppo non può essere che temporaneo, senza che si possa escludere il loro comportamento esofilo.

Le ricerche effettuate hanno permesso di stabilire che durante la stagione secca sono soprattutto *A. gambiae* forma tipica ed *A. gambiae* var. *melas*, grazie ai focolai larvali di fortuna, a mantenere ed assicurare la trasmissione della malaria. La forma *melas* è stata trovata infettata dall'ematozoo, e le due forme di *gambiae* sono in questa regione molto antropofile. Esiste in questa regione, durante la stagione secca, e specie nella capitale della Guinea, una discordanza tra anofelismo e malaria che si spiega con il comportamento speciale di *A. gambiae*.

Nel lavoro sono descritti i focolai larvali di fortuna di queste due specie. La loro presenza spiega la trasmissione della malaria nella stagione secca ed anche la dispersione delle anofeline nella stagione delle pioggie quando compaiono i focolai temporanei.

Benchè il grado di infezione malarica sia diminuito dopo l'applicazione di insetticidi ad azione residua, nella città di Conakry si hanno ancora casi di malaria autoctona. Nella stessa città è stata ugualmente messa in evidenza l'esistenza di malaria congenita.

Dai risultati ottenuti (che si rivelano incompleti) emerge che la lotta antimalarica deve assumere forme variate a seconda della natura del terreno, e che si deve di preferenza, senza abbandonare la distruzione degli insetti adulti a mezzo degli insetticidi ad azione residua, far ricorso agli antichi procedimenti di soppressione dei focolai permanenti o potenziali, vale a dire a lavori antilarvali permanenti.

MALARIA AND THE OUTLOOK FOR THE ANTIMALARIAL CAMPAIGN IN LOWER GUINEA.

This article represents a compendium of the enquiries made by the author, during two missions, on malaria and anophelism during the dry season in Guinea and notes the permanent and potential foci of anophelines in East Africa in the litoral zone of the Kaloum peninsula and the estuary of the Rio Nunez.

Data are reported from a survey of anopheline species and some indications of the extent of malarial infection.

In was found that during the dry season several species of anophelines were present, especially in the larval stage, in the Kaloum peninsula and the Rio Nunez region, but the greater part of these did not truly represent the fixed autocthonous fauna. They had probably been introduced into the Kaloum peninsula from Middle and Upper Guinea and do not adapt to this dry region where their development can only be of a temporary nature although their exophilic behaviour can not be excluded.

The studies performed showed that during the dry season the transmission of malaria is maintained and assured by, above all, the typical form of *A. gambiae* and *A. gambiae* var. *melas* by means of adventitious foci of larvae. The *melas* form was found infected with heamatozoites and both forms of *gambiae* are highly anthropophilic in this region. In this region in the dry season and especially in the capital of Guinea there is a discordance between anophelism and malaria which can be explained by the special behaviour of *A. gambiae*.

The adventitious larval foci of these two species are described in the article. Their presence explains the transmission of malaria in the dry season and also the dispersion of anophelines in the rainy season when short-lived foci appear.

Although the level of malaria has diminished after the application of residual acting insecticides in the city of Conakry, there are still cases of autocthonous malaria. Congenital malaria has also been demonstrated in this city.

From the incomplete results obtained it emerges that the antimalarial campaign must assume various forms depending on the nature of the terrain and that without abandoning the destruction of the adult insects by residual insecticides, recourse must be made to the older procedures of suppression of permanent or potential foci in order to bid farewell to permament antilarval work.

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NOTE SUR LES CULICIDÉS DANS LA VALLÉE DE LA LUFIRA (1941-1942)

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Après l'étude des conditions extérieures de la plaine alluviale traversée par la Lufira entre Kapolowe et les Chutes Cornet dans le Haut-Katanga, les gîtes larvaires ainsi que l'éthologie des adultes mansoiniodes ont été définis dans cette région.

Dans la présente note nous avons l'intention de résumer les résultats d'observations faites dans une région marécageuse du Haut-Katanga, région comprenant un barrage dont la construction fut achevée en 1930 et qui fut rehaussé de 0,75 m. en 1938, de 2,50 m. en 1939.

La région est située par 27° de longitude ouest et 11° de latitude sud. Les observations ont été faites depuis le 24 mars 1941 jusqu'au 30 août 1942. Malgré le long délai entre cette époque et la présente publication, nous croyons qu'elle présente encore un caractère d'originalité.

La Lufira coule entre Kapolowe et les chutes Cornet (**) dans une large plaine alluviale d'environ 1.110 m. d'altitude. La surface de cette plaine est de ± 400 Km². Sa dénivellation est de 3 m. Nous avons trouvé dans la plaine et les rivières et marais environnants:

1) A l'état submergé:
Potamogeton sp.; *Utricularia* div. sp. (cfr. *U. Thonningii* et *U. stellaris*); *Chara* sp; Algues diverses.

2) A l'état flottant:
Diverses Nympheacées: cfr. *Nymphaea lotus, N. divaricata;* Convolvulacées: *Ipomea aquatica;* Divers *Polygonum*: *P. salicifolium, P. tomentosum; Azolla* sp.; *Riccia* sp.

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(**) Endroit de la construction du barrage.

3) Dans les marais roseliers et prairies inondées:

Graminés. *Leersia hexandra* Sw.; *Oryza barthii; Digitaria* sp.; *Brachyaria kostchiana.*

Cyperacées. *Cyperus torulinium; Mariscus longibracteatus; Mariscus laxiflora; Fimbristylis* sp.

Polygonacées. Divers *Poligonum.*

Typhacées. *Typha latifolia.*

4) Dans les savanes paludicoles, sur les bords marécageux des rivières et dans les îles flottantes:

Graminées. *Cleistachne sorghoïdes; Jardinea* sp.; *Echinochloa colonum* Link; *Hyparrhenia rufa; Hypparrhenia cymbaria; Hyparrhenia speciosum; Setaria sphacelata; Setaria aurea; Andropogon* sp.; *Rootboellia exaltata* L. F.; *Arthraxon quartinianus* (A. Rich) Nash.

Cyperacées. *Kyllingia cylindrica; Kyllingia alata; Fuirena umbellata; Fuirena muricathus; Fuirena macranthus; Cyperus fulgens; Scirpus muricinus.*

Malvacées. *Hibiscus* sp.

Oenotheracées. *Jussieua* div. sp.

Rubiacées. *Oldenlandia* sp.

Commelinacées. *Floscopa glomerata; Floscopa rivularis; Commelina scaposa.*

Polygonacées. *Polygonum salicifolium; Poligonum serulatum; Poligonum tomentosum; Polygonum speciosum.*

Amaranthacées. *Alternanthera nodiflora.*

Ombellifères. *Hydrocotyle centella*

Compositacées. *Gynura* sp.

C'est sans doute à partir de 1939 que des modifications profondes de la flore ont eu lieu dans le bassin de retenue. Nous y avons trouvé en mars 1941 une prairie inondée, immense, peuplée en général sur les bords: côté ouest de roseaux (*Cyperus* et *Typha*), côté est et sud de *Leersia.*

Ceci constituait une bande relativement mince, le restant de la surface était occupé par des *Oryza.* Il existait néanmoins à l'intérieur de cette zone des îlots flottants à *Leersia* et d'autres à enchevètrement de roseaux et même d'une flore plutôt terrestre. A certains endroits l'eau était relativement libre ou recouverte de nympheacées.

Les trajets des rivières inondées étaient souvent marqués par un ruban de *Typha* et *Cyperus* flottants.

Nous avons étudié spécialment ce bassin, mais aussi les rivières tributaires et marais qui n'ont pas subi l'influence du barrage.

Ces derniers nous donnaient une idée de l'aspect de la plaine alluviale avant la construction du barrage.

Météorologie

La région jouit d'un climat tropical tempéré par l'altitude. En général il existe une saison sèche et froide d'avril à octobre et une saison des pluies de novembre à mars.

La moyenne des précipitations atmosphériques pour une période antérieure de 10 ans est de 1103,9 mm. Pendant la période de nos observations (1941-42) le total des pluies a été de 956,7mm.

L'humidité relative et le déficit de saturation sont en relation avec les saisons.

La température (période 1941-42). Les températures moyennes mensuelles oscillent entre 20° et 26°. Les maxima mensuelles absolues entre 30° et 35° Il n'est pas sans intérêt de donner les minima absolues parce qu'elles jouent un rôle important dans la biologie des moustiques adultes.

1941							1942				
juin	juillet	août	sept.	octob.	novem.	déc.	janvier	février	mars	avril	mai
7,6	6,6	9,1	13,4	13	16,6	16,8	16	16,1	15,1	10,5	9,5

Les vents. Pendant la saison sèche les vents soufflent en compagnie de l'alizé du sudest, du secteur est.

La variation de la direction a été très faible de juin à octobre 1941 et de février à avril 1942.

Pendant la saison des pluies, le vent venait de toutes les directions et celles-ci variaient d'un endroit à l'autre.

La vitesse du vent dépasse souvent la marque 3 de l'échelle Beaufort et oscille autour de la marque 1.

Le niveau des eaux. Le niveau des eaux aux chutes Cornet oscillait de 1931 à 1936 autour de la côte conventionnelle de 206 m., de 1937 à 1938, entre 206, 79 et 203, les niveaux le plus bas étant atteints vers le mois d'octobre (fin saison sèche), de 1939 à 1941 les niveaux suivants on été observés:

1939	janvier	204
	juin	208,35
	décembre	206,40
1940	juin	209,20
1940-41	décembre à mars	208,50
1941	avril-mai	209,25
1941	novembre	207,85
1942	avril	208,97

Lorsque les eaux atteignent la côte 206, on peut considérer la plaine comme vidée. Ceci se passait avant 1939 à la fin de la saison sèche et toute la plaine subissait l'influence des feux de brousse.

Depuis 1939, le réservoir ne se vidait plus et la période des eaux les plus basses était observée vers les mois de décembre-janvier (saison des pluies).

Espèces de moustiques rencontrés

A l'*état larvaire*:

Mansonioides (*Taeniorhynchus*) *africanus* Theo.
M. uniformis Theo.

Anopheles gambiae Giles
A. funestus var. *leesoni* Evans
A. funestus var. *rivulorum* Leeson
A. funestus var. *confusus* Ev. and Lees.
A. coustani Lav.
A. coustani var. *ziemanni* Grün.
A. squamosus Theo.
A. squamosus var. *cidyppis* de Meillon.
A. rufipes Gough
A. pretoriensis Theo.
A. natalensis Hill and Hayden
A. demeilloni Ev.
A. seydeli Edw.
A. maculipalpis Gil.
A. nili Theo.
A. marshalli Theo.
A. moucheti Ev.
A. theileri Edw.
A. distinctus News. and Cart.

Culex guiarti Blanch.
C. horridus Edw.
C. bitaeniorhynchus Gil.
C. inconspicuosus Theo.
C. grahami Theo.
C. perfidiosus Edw.
C. duttoni Theo.

Culex poecilipes Grandpré
C. tigripes Grandpré
C. annulioris Theo.
C. salisburiensis Theo.
C. univittatus Theo.
C. decens Theo.
C. chorleyi Edv.
C. macfiei Edw.

Il n'a été possible d'exprimer les proportions de moustiques à l'état *larvaire* que pour le sous-genre *Mansonioides*. Pour ceux-ci un système relativement simple a été adopté:

L'on prélevait 5 poignées d'herbes que l'on agitait dans un seau à double tamis et l'on comptait ensuite les larves ainsi récoltées. L'indice dont on parlera plus tard est calculé sur 10 seaux.

Il a été ainsi identifié 9.834 larves de *Mansonioides*, dont 9.407 *uniformis* et 407 *africanus*, soit respectivement 95,7% et 4,3% La même chose aurait pu être faite pour le sous-genre *Coquillettidia*, mais comme 2 larves seulement avaient été décrites, il n'était pas possible de préciser l'espèce.

Nous avons toutefois pu établir la proportion de *Mansonioides* et de *Coquillettidia*. Sur 1638 larves du genre *Taeniorhynchus* il y avait 509 *Mansonioides* et 561 *Coquillettidia*.

A l'*état adulte*:

926.027 moustiques on été identifiés parmi lesquels;
893.030 *Mansonioides* (*Taeniorhynchus*);
535 *Coquillettidia* (*Taeniorhynchus*);

15.935 Anophèles;
16.527 Culicines.

Quelques identifications plus précises ont été faites: pour les *Mansonioides* il a fallu examiner les extrémités génitales des femelles.

Mansonioides déterminés: .	3.900	*Anopheles*:	11.205
M. uniformis Theo . . .	3.500	*A. funestus* Gil.	8.827
M. africanus Theo. . . .	400	*A. coustani* var. *ziemanni* Grün.	1.538
Coquillettidia:	643	*A. coustani* var. *tenebrosus* Don.	44
C. flavocinctus Edw. . . .	500	*A. gambiae* Gil.	603
C. microannulatus Theo. .	71	*A. distinctus* News. & Cart.	67
C. aurites Theo.	40	*A. coustani* Lav. . . .	67
C. nigrithorax Theo . . .	13	*A. squamosus* Theo. . . .	50
C. cristatus Theo.	8	*A. pretoriensis* Theo. . .	6
C. maculipennis Theo . . .	6	*A. moucheti* Ev.	3
C. fuscopennatus Theo. . .	3		
C. metallicus Theo.	1		
C. annetti Theo.	1		

Parmi les autres moustiques rencontrés mentionnons:

Culex univittatus Theo.
C. duttoni Theo.
C. bitaeniorhynchus Gil.
C. poecilipes Grandpré.
Aëdomya sp.
Aedimorphus sp.
Megarhinus sp.
Erethmapodites sp.
Aëdes sp.

Une nouvelle variété d'anophèles notamment *A. coustani* var. *caliginosus* de Meillon fut reconnue dans cette région.

* * *

L'immense majorité des moustiques appartient au genre *Taeniorhynchus*. A l'état larvaire *Coquillettidia* et *Mansonioides* semblent s'équilibrer mais par contre à l'état adulte la majorité est représentée par le sous-genre *Mansonioides*.

En réalité il y avait eu une invasion de ces moustiques à partir de 1940 qui revêtait le caractère d'un véritable fléau, dont nous n'avons vu que la fin. Il nous a été possible, néanmoins, de nous livrer à quelques observations intéressantes sur le groupe *Mansonioides* et de nous imaginer ce qui s'était passé l'année précédente.

Larves.

On sait depuis les travaux d'Ingram (1912) et de Da Costa-Lima (1915) puis plus tard de Mac Fie (1917) que les larves du genre *Taeniorhynchus* s'at-

tachent aux radicelles des plantes acquatiques et respirent aux dépens de l'oxygène se trouvant dans les espaces intercellulaires. Pendant un certain temps la seule plante reconnue comme support était *Pistia stratiotes*, mais dans la suite bien d'autres plantes ont été décrites parmi lesquelles il n'est pas sans intérêt de mentionner *Eichornia crassipes*.

Nous-mêmes avons trouvé dans la région étudiée des larves de *Mansonioides* sur le plantes suivantes, dont plusieurs avaient été décrites comme positives auparavant:

Oryza barthii; Leersia hexandra; Alternanthera nodiflora; Polygonum salicifolium; Polygonum tomentosum; Floscopa glomerata, Fl. rivularis; Cyperus torulinium; Echinochloa colonum; Oldenlandia sp.; *Arthraxon quartinianus; Jussieua* sp. (plusieurs); *Hyparrhenia cymbaria, Hyp. rufa, Hyp. speciosum.; Commelina scaposa; Typha latifolia; Setaria sphacelata; Hibiscus* sp.; *Pycreus macranthus*.

Il ne paraît donc pas y avoir de spécificité en cette matière. Par contre l'attention avait été attirée en 1934 par Rosenwald sur le fait que les élevages de *Mansonioides annulifera* réussissaient dans de très mauvaises conditions pourvu que l'extrême voracité des larves fût satisfaite, ce qui était réalisé par l'addition d'excréments de cobayes.

En 1938, Iyengar démontre la nécessité pour les larves de bénéficier dans l'eau du gîte d'une certaine concentration en matières organiques. Même sur *Pistia* lorsque la nourriture est insuffisante, l'on ne trouve pas de larves, fait que nous avons observé en 1931 dans le Stanley Pool. Pour Iyengar, les gîtes les plus appropriés sont ceux qui contiennent de l'eau polluée par des noix de coco et par des excréments humains ou d'animaux.

En 1939, Bonne-Wepster et Brug trouvent que les larves de *Mansonioides* ne vivent pas tellement fixées sur les radicelles, mais bien dans la vase sous-jacente.

Nous avons été amenés par conséquent à étudier la formation de la vase.

La vase se produit exclusivement dans l'eau aux dépens de matières organiques en suspension ou dissolution (par ex: parties de plantes submergées en voie de décomposition, cadavres et excréments d'animaux, plancton mort).

Le mode de formation de la vase diffère suivant que le milieu est riche en oxygène (les agents sont des microbes aërobies) ou qu'il soit pauvre en oxygène (microbes anaërobies).

On peut considérer que dans le bassin de retenue contenant de l'eau stagnante il y aura plutôt formation de vase par anaërobiose (réduction) et dans les rivières par aërobiose (oxydation).

P. Van den Brande pédologue au comité spécial du Katanga a reconnu dans la vase spéciale du bassin un *sapropel*, vase fluide, pâteuse et fétide.

Au cours de nos observations, nous avons choisi un grand nombre de

biotopes dont les uns étaient prospectés régulièrement et les autres, trop difficiles d'accès, occasionnellement.

Nous avons dit qu'un grand nombre de plantes servait de support aux larves de *Taeniorhynchus*: cependant dans la prairie inondée du réservoir, la majorité des graminées était *Leersia* ou *Oryza*. C'est donc sur ces 2 graminées que nous avons fait en ordre principal nos observations.

Ces Graminées commencent par pousser en terre ferme et peuvent, en raison des crues, s'allonger démesurément. *Leersia* atteint 1 m. 80 et *Oryza* 2 m. 40.

La crue évolue par poussées brusques et violentes et il y a donc beaucoup plus d'agitation de l'eau et d'oxygène qu'à la décrue. Lorsque la décrue s'installe, les tiges s'affaissent et se replient sur elles-mêmes et il n'émerge plus de l'eau que les inflorescences. Plus tard la tige principale meurt mais la plante repousse par stolons. A ce stade le marais présente un enchevêtrement de stolons, de radicelles, de chaumes pourris, le tout chargé de vase fétide (sapropel). Le matelas ainsi formé peut reposer sur le fond et, en temps de crue, flotter entre deux eaux lorsque les racines le retiennent encore ou enfin faire surface.

Ceci constitue le milieu favorable à la prolifération des larves de *Mansonioides*.

Lorsque les eaux sont au plus bas, il se présente:

1) une zone asséchée depuis plusieurs mois, le matelas y disparaît et de nouvelles plantes poussent ainsi en terre ferme;

2) plus au large, ce matelas est seulement exondé, mais persiste néanmoins jusqu'à la crue suivante;

3) enfin, plus loin encore, il peut rester flottant pendant toute l'année.

Lors de la crue suivante le même cycle recommence pour les graminées ayant poussé en terre ferme (catégorie 1°).

Pour les catégories 2) et 3), les racines du chaume primitif pourrissent. En certains endroits les radicelles des stolons peuvent se fixer $\pm$ lâchement au sol. Efin encore plus au large, celles-ci ne se fixent pas et lors de la poussée des eaux, les nouvelles plantes ainsi que leur matelas sous-jacent se mettent à flotter à la surface de l'eau.

Mais ici il y a une nette différence entre *Oryza* et *Leersia*. Tandis que ces derniers peuvent repousser et fructifier à l'état flottant, les *Oryza* meurent. On aperçoit alors finalement à la surface une croûte en décomposition qui finit par sombrer. Il se crée ainsi d'énormes espaces libres et qui resteront tels pour autant qu'un ensemencement au sol devienne impossible ou que des îles flottantes ne s'y accumulent pas. En eau moins profonde se présentent des populations de Nymphéacées.

Voici quelques chiffres au sujet de la densité des larves en rapport avec les divers biotopes. Les observations méthodiques ont eu lieu de novembre

1941 à juillet 1942. Les eaux ont commencé à baisser à partir de mai 1940 pour atteindre leur niveau le plus bas en décembre 41. Les eaux ont remonté à partir de la 3ème semaine de janvier pour atteindre un maximum en avril 42, pour décroître ensuite à partir de mai 42.

1) Une première bande à Leersia a été asséchée complètement et le matelas a disparu; les *Leersia* ont repoussé en terre ferme. Cette région a alors été inondée à partir de mars et le gîte reste négatif jusqu'à fin avril. Dès ce moment, le taux des larves augmente pour atteindre en juin un indice de 17 larves pour 10 seaux et en juillet de 11. Au total, il a été mesuré 150 seaux où on a trouvé 91 *Mansonioides* soit un indice de 6.

2) Dans une autre bordure de végétation, les *Leersia* ont été exondés ou non mais pas suffisamment pour que le matelas disparaisse. Les gîtes sont négatifs pendant les 2 premières semaines de novembre 41. Au total pour 1717 seaux on trouve 1300 *Mansonioides*, soit un indice de 7,7.

Pendant les 3 dernières semaines de juillet on trouve 190 larves pour 65 seaux, soit un indice de 30.

3) Ailleurs le matelas de *Leersia* est recouvert avec des dépôts de sable sans vase; ils ont été observés depuis mars jusque juillet: 70 seaux donnent 7 larves, soit un indice de 0,4.

4) Ailleurs encore, les *Leersia* flottent sur un matelas sans vase. Sur 208 seaux il y a 10 *Mansonioides* soit un indice de 0,4.

La plaine à *Oryza* nous donne des résultats semblables:

1) *Oryza* s'étant exondé ou non, mais ayant conservé son matelas (avec vase): 3466 seaux, 3422 *Mansonioides* soit presque 10 comme indice.

2) *Oryza* flottant sans vase donnent d'après le degré de décomposition les indices suivants:

327	seaux	donnent	0,8
129	»	»	1,9
242	»	»	0,7

Nous avons effectué de très nombreux prélèvements dans les îles flottantes. Celles-ci flottent sur un enchevêtrement de stolons et de débris: elles ne contiennent pas de vase; aucune larve de *Mansonioides* n'y a été trouvée.

Dans les rivières marécageuses, nous avons effectué 11.251 prélèvements qui nous donnent un indice larvaire de 4,3.

Les biotopes de ces rivières sont très complexes, tantôt ce sont des marais roseliers presque purs, tantôt des rivières herbeuses à eau plus ou moins courante; le plus souvent on y trouve un mélange de Cyperacées, Typhacées, *Leersia, Polygonum* et *Oryza*.

Spécialement dans les marais roseliers faisant partie de la région inondée, on a trouvé, pour 841 seaux, un indice de 5,9.

Ces indices sont donc plus bas que ceux de la prairie inondée pour 2 rai-

sons; sans doute parce les *Typha* et *Cyperus* ne présentent pas cet enchevêtrement de stolons et ensuite parce qu'il s'agit d'eau plus aérée où la vase se forme plutôt par oxydation.

Pour résumer, voici, selon nous, les conditions qui doivent être remplies pour l'obtention d'un gîte larvaire favorable; température adéquate (la température de l'eau varie entre 26° et 15° C.), présence de vase et d'un enchevêtrement de stolons. Ces conditions seront le mieux réalisées pendant la décrue, mais surtout lorsque celle-ci est faible et a lieu en saison chaude.

Il s'ensuit que d'après les interférences des saisons et des crues, le gîte favorable du bassin de retenue peut couvrir de 30 à 400 Km². Le plus grande surface de gîte larvaire a sans doute été obtenue depuis juin 1940 à février 1941, période de la grande invasion d'adultes. Nous avons en mars 1941 trouvé la prairie au stade de matelas semi- flottant, les graminées en pleine fructification, mais la vase était absente, les mansonioides aussi; les crues venaient de s'installer.

On sait aussi que la plupart des espèces d'Anophèles exigent de l'eau aérée, c'est ainsi que par ex. *A. nili, A. funestus* sont trouvés dans des eaux à courant extrêmement rapide, d'autres anophèles sont à ce sujet moins exigeants, tels que *implexus, gambiae, maculipalpis.*

Nous n'avons pas trouvé une seule larve d'Anophèle dans le gîte à *Mansonioides.*

Moustiques (Mansonioides) adultes.

Pour essayer de comprendre l'éthologie de ces moustiques, nous avons eu recours à diverses méthodes de capture:

1) le *piège* de Magoon. Nous avons placé 7 de ces pièges dans lequel l'appât était un mouton. Ces pièges étaient éloignés du réservoir de 0 à 15 Kms.

2) Capture sur *homme dehors*: 35 stations.

Ces captures sont entachées d'erreurs car il arrivait que les assauts de moustiques étaient trop violents pour qu'on puisse capturer tout ce qui piquait.

3) Capture dans les *cases* le jour: dans le même nombre de stations.

Tout d'abord, et ceci concorde avec les observations de la plupart des auteurs, les *Mansonioides* présentent un degré marqué d'hygrophilie et d'exophilie. Alors que dans un piège on peut capturer des milliers de moustiques par nuit, c'est par dizaines seulement que l'on compte ceux capturés dans les cases. Ce fait est confirmé par de nombreuses observations faites sous la tente. Nous avons remarqué fréquemment que notre tente était tapissée de mansonioides au milieu de la nuit et que ceux-ci quittaient vers 4h. du matin.

La période d'invasion et de grande prolifération des adultes correspond avec la grande production de larves dans le bassin de retenue. En effet, des endroits fort éloignés (jusque 15 kms) étaient envahis pendant cette période alors que les gîtes locaux ne donnaient que faiblement.

Ceci pour autant q'il s'agisse de saison chaude. Pendant celle-ci l'attaque a lieu toute la nuit. Par contre durant les mois plus froids, les moustiques ne commencent à piquer qu'après le lever du soleil jusque vers 9h. du matin et au crépuscule du soir. Ceci ne se passe alors que à proximité du gîte. Donc, même en cas de grosse production larvaire, en saison froide, les *Mansonioides* s'éloignent très peu de leur gîte et les villages ne sont pas atteints.

Les attaques des *Mansonioides* se font par vagues irrégulières par ex. 1 piège donne du 27 décembre au 4 janvier 1942: 92, 225, 837, 3084, 3437, 2235, 8159, 60; du 16 au 21 janvier: 4217, 16.264, 1970, 2812, 2899, 5976.

Ceci ne peut être expliqué que par les circonstances microclimatiques locales et surtout la vitesse du vent: ci-dessous quelques résultats de captures nocturnes sur homme

le 9/10/41

Heures	17	18	19	20	21	22	23	24	1
vitesse (Beaufort)	2	2	1	1	0	3	4	4	5
moustiques	0	8	94	105	195	10	10	7	2

le 24/10/41

Heures	17	18	19	20	21	22
vitesse	2	4	2	0	0	0
moustiques	0	2	342	614	593	761

le 1/5/42

Heures	19	20	21	22	23	24	1	2	3	4	5
vitesse	2	2	2	4	4	3	3	3	2	2	1
moustiques	169	199	106	97	82	65	69	71	35	27	42

Dès que la vitesse dépasse la marque 2 les moustiques diminuent considérablement. Par contre une pluie fine ne met nullement à l'abri des moustiques.

Distance de vol des moustiques et influence de la direction du vent.

Nous ne disposions évidemment pas à l'époque d'isotopes radio-actifs; mais nous avons adopté la méthode par poudrage (poudre impalpable d'imprimerie Majid). 4 pièges de Magoon ont été utilisés comme émetteurs. Au matin ces pièges étaient entourés d'un calicot de protection et les moustiques étaient poudrés au moyen d'un appareil à fly-tox. Le piège restait ensuite ouvert jusqu'au soir. Pour les 4 pièges nous avons employé 4 poudres de couleurs différentes. L'expérience a duré du 5-12-41 au 30-5-42. Afin d'avoir une idée du nombre de moustique ainsi lâchés, nous avons à intervalles réguliers laissé les pièges se remplir. Nous avons sacrifié et compté les moustiques qui s'y trouvaient et estimé qu'environ un demi-million de moustiques colorés avaient été lâchés.

Nous avons dans une cinquantaine de postes, recapturé ½ million de

moustiques. Sur ce ½ million 704 étaient colorés. Ils se distribuaient comme suit:

235	de	0	à	5	kms
430	»	5	»	10	»
39	»	10	»	15	»
0	»	15	»	20	»

Cette aire de dispersion correspond bien à celle établie plus grossièrement par l'estimation de la densité seule.

Il n'a pu être établi durant ces 6 mois aucune relation entre la direction des vents et l'invasion des *Mansonioides*. Grâce aux 4 couleurs différentes employées nous avons pû établir que, dans ce rayon de 15 Kms., des moustiques venaient de toutes les directions.

Reste à expliquer comment ces moustiques peuvent se disperser aussi loin à partir de leur gîte. Nous avons fréquemment observé, au cours de nos prospections, le phénomène suivant; lorsqu'on marche à des distances considérables de tout gîte, on est fréquemment assailli même en plein jour par des *Mansonioides*, mais cela uniquement aux bords de ruisseaux et fonds humides qui eux-mêmes ne constituent pas de gîtes possibles. Nous pensons par conséquent que les *Mansonioides* s'infiltrent le long des endroits humides et rivières et manifestent leur activité lorsque les conditions idéales de température et d'humidité sont remplies.

En résumé, les *Mansonioides* vivent à l'état larvaire dans des biotopes spécialisés. Ils peuvent respirer aux dépens de n'importe quelle plante aquatique à condition que celles-ci aient de nombreuses radicelles dans une eau chargée de sapropel. Les adultes sont exophiles, extrêmement sensibles au vent. aux températures basses et à la sécheresse.

Les déterminations de plantes ont été faites en 1941 par Monsieur QUARRÉ botaniste du Comité Spécial du Katanga (C.S.K.) et par le professeur PHILIPS de l'Université du Witwatersrand-Johannesburg.

Nous a aidé de ses conseils P. VAN DEN BRANDE, pédologue du C.S.K.

Le professeur BOUILLENNE, directeur de l'Istitut de botanique de Liège, a bien voulu revoir la texte de la présente note.

La Société Générale des forces hydroélectriques du Katanga (Sogefor) a subsidié cette mission.

Enfin, ce travail n'aurait pû être réalisé sans le dévouement et le courage de messieurs SELIMANI MBWANA et MAYOLA SADI, gardes sanitaires.

Nous les en remercions tous vivement.

NOTE SUI CULICIDI NELLA VALLE DELLA LUFIRA (1941-1942)

Sono state studiate la flora, le condizioni metereologiche, i venti, il livello delle acque ed i culicidi della pianura alluvionale attraversata dalla Lufira tra Kapolowe e le Cascate Cornet nell'Alto Katanga.

In base alle osservazioni fatte, si è potuto constatare che i *Mansonioides*, che costituiscono la maggioranza dei culicidi della zona, vivono allo stato larvale in biotopi specializzati. Essi possono respirare alle spese di qualsiasi pianta acquatica, purchè provvista di numerose radicelle, in un'acqua carica di sapropel.

Gli adulti sono esofili, estremamente sensibili al vento, alle basse temperature ed alla siccità.

NOTE ABOUT THE CULICIDAE OF THE LUFIRA VALLEY (1941-1942).

The flora, the meteorological conditions, the winds, the level of the water and the culicidae of the alluvial plain of the Lufira-river between Kapolowe and the Cornet-falls in the High Katanga, have been studied.

According to these observations, it has been stated that Mansoniodes which constitute the majority of the culicidae in this place, live in the larval stage in specialised biotopes. They take their air from any aquatic vegetation, provide it shows abundant rootlets loaded with sapropel.

The adults are exophilic, very sensitive to wind, low temperature and drought

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ÜBER SCHWANKUNGEN IN DER ZUSAMMENSETZUNG NATÜRLICHER ANOPHELES-POPULATIONEN IN DEUTSCHLAND

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In Verbindung mit neueren Beobachtungen über *Anopheles* in Deutschland werden genauere Zahlen über die Zusammensetzung der Population an 2 Biotopen in Norddeutschland gebracht, an welchen *A. atroparvus* und *A. messeae* vorkommen. Im Verlauf einer über 25 Jahre dauernden Beobachtungszeit ist hier die Population gleich geblieben. Das Verschwinde der letzten endemischen Tertiana in Deutschland hängt nicht mit einer Zunahme von *A. messeae* auf Kosten von *A. atroparvus* zusammen. Die Anophelen-Dichte ist ganz allgemein in den letzten Jahren stark zurückgegangen.

Vorbemerkungen.

Die europäische Malaria wird bald der Geschichte angehören. Das gilt nicht nur für Nord- und Mittel-, sondern auch für Südeuropa. Nach dem letzten Kriege war es in Deutschland an verschiedenen Stellen zu einer autochthonen Malaria gekommen, die mehrere Tausend Fälle betraf, 1946 ihren Höhepunkt erreichte und bis 1950 wieder abklang. Im gleichen Jahre wurden auch die letzten 3 Fälle der von dieser Situation ganz unabhängigen endemischen Tertiana in Ostfriesland registriert (Weyer 1956). Seitdem sind weder hier noch an anderen Stellen in Deutschland weitere oder neue Malariafälle bekannt geworden. Auch bei der nordholländischen Malaria, die der ostfriesischen benachbart ist, zeigte die Morbiditätskurve seit 1949 einen stetigen Abfall (Kraan 1955). Bemerkenswert ist, dass die norddeutsche Malaria im Unterschied zu der in anderen Ländern von selbst verschwunden ist. Massnahmen zur Bekämpfung der Anophelen sind in Deutschland nur vereinzelt

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nach dem Kriege durchgeführt worden; sie haben auf die Malaria- und Mükkenlage keinerlei Einfluss gehabt.

Diesem Umstand ist es zu verdanken, dass die Anophelesfauna in Deutschland heute noch mehr oder weniger ihren ursprünglichen Charakter besitzt. Daher bot sich die günstige Gelegenheit, im Rahmen der Resistenzforschung in Deutschland noch mit Freilandpopulationen von *Anopheles* zu arbeiten, die niemals mit Insektiziden in Berührung gekommen waren. Von dieser Möglichkeit ist u. a. bei Untersuchungen Gebrauch gemacht worden, welche die Empfindlichkeit von Freilandmücken für DDT in Abhängigkeit vom Jahreszyklus betreffen (GARMS, WEYER & REHM 1959). Dabei konnten auch gleich weitere Beobachtungen über die Zusammensetzung der *Anopheles*-Populationen und ihre Aederungen gesammelt werden, die schon früher Gegenstand von Untersuchungen waren (WEYER 1939, 1951). In Verbindung mit einem Bericht über diese Untersuchungen sollen auch einige neuere, von anderer Seite stammende und z. T. noch nicht veröffentlichte Beobachtungen über Anophelen in Deutschland erwähnt werden.

NEUE BEOBACHTUNGEN ÜBER DIE ANOPHELESFAUNA IN DEUTSCHLAND.

Nach BAER (1960) ist in Thüringen — die Untersuchungen wurden von 1952 bis 1955 durchgeführt — *Anopheles messeae* Falleroni mit 50% am häufigsten. An zweiter Stelle steht *A. typicus* Missiroli & Hackett, der mit *A. messeae* gemeinsam vorkommt, aber in höheren Lagen auch reine Populationen bildet. 41 (2%) der gefangenen und genauer determinierten Weibchen waren *A. atroparvus* van Thiel. Diese Mücken stammten von 4 verschiedenen Plätzen, die in der Nähe von Salzstellen lagen. In den Flussniederungen, in denen ein hoher Feuchtigkeitsgehalt herrscht, gehörten 6 % der Fänge zu *A. claviger* Meigen (= *bifurcatus* Meigen). An 10 Plätzen wurde *A. plumbeus* Stephens (= *nipripes* Staeger) nachgewiesen. Unter den im Freien beim Stechen am Vieh oder Menschen gefangenen Mücken überwogen *A. messeae* und *A. claviger*.

KÜHLHORN (1958a) wies Larven von *A. messeae* und *A. typicus* in Oberbayern bis zu einer Höhe von 775 m nach. Der höchste Punkt, an welchem noch *A. claviger* vorkam, lag bei 1. 377 m. Für *A. claviger* wirken sich warme und trockne Sommer ungünstig aus, da ein Teil der Brutplätze verschwindet und die feuchtigkeitsarme Luft auch den Imagines abräglich ist (KÜHLHORN 1959). Stark besonnte Gewässer sind als Brutplätze ungeeignet, weil die Larven schon bei einer länger anhaltenden Durchschnittstemperatur von 20°C an der Wasseroberfläche geschädigt werden. KÜHLHORN (1958b) stellte auch fest, dass sich die Larven von *A. claviger* vorwiegend von Algen ernähren, und nimmt an, dass chemische und physikalische Eigenschaften der Brutgewässer indi-

rekt über das Phytoplankton auf das Vorkommen der Larven Einfluss haben.

Am Niederhein bei Krefeld und Kaldenkirchen fand KNOTT (1959) nur *A. maculipennis* und *A. claviger*. Soweit die Angehörigen des *maculipennis*-Kreises bestimmt werden konnten, handelte es sich um *A. messeae*. Anophelen wurden im Vergleich zu anderen Stechmücken nur in geringer Zahl gefunden. *A. claviger* hat in der dortigen Gegend 2 Generationen im Jahr. Die Larven

TAB. 1.

Zusammensetzung der Anopheles-Population an zwei Biotopen in Norddeutschland. Bestimmung nach den Gelegen.

Beobachtungszeit	Zahl der Gelege (%)	
	A. atroparvus	*A. messeae*
	a) *Ostfriesland*	
1932	187 (91.7)	17 (8.3)
1933	41 (100.0)	
1936	63 (86.3)	10 (13.7)
1937	39 (86.7)	6 (13.3)
1938	108 (99.1)	1 (0.9)
1949	79 (84.1)	15 (15.9)
1950	38 (95.0)	2 (5.0)
1955	201 (96.2)	8 (3.8)
1957	248 (95.1)	13 (4.9)
1958	182 (91.5)	17 (8.5)
1959	113 (83.7)	22 (16.3)
zusammen	1299 (92.1)	111 (7.9)
	b) *Elbmarschen*	
	10 (16.1)	52 (83,9)
1931	13 (31.7)	28 (68.3)
1932	27 (29.3)	63 (70.7)
1933	16 (16.8)	79 (83.2)
1934	77 (47.2)	86 (52.8)
1936	81 (59.6)	55 (40.4)
1937	231 (90.6)	24 (9.4)
1939	167 (72.6)	63 (27.4)
1940	24 (63.2)	14 (36.8)
1944	14 (31.8)	30 (68.2)
1946	69 (65.1)	37 (34.9)
1949	26 (72.2)	10 (27.8)
1950	23 (75.0)	7 (25.0)
1955	17 (4.0)	411 (96.0)
1957	9 (7.5)	111 (92.5)
1958	42 (38.9)	66 (61.1)
1959		
zusammen	846 (42.7)	1136 (57.3)

fanden sich teilweise zusammen mit denen von *Theobaldia morsitans* Theobald un *Aedes diversus* Theobald. Im Rahmen einer Untersuchung über Biologie und Oekologie der Culiciden im Gebiet von Frankfurt/Main wies Scherpner (1959) *Anopheles atroparvus, A. messeae, A. typicus, A. claviger, A. plumbeus* und *A. algeriensis* Theobald nach. Für die Bestimmung von *A. atroparvus* und *A. messeae* wurde ausser den Gelegen auch der Schuppenindex (Laven 1950) benutzt. Die Methode bewährte sich, so dass damit in den meisten Fällen auch Larven nach der Aufzucht zur Imago determiniert werden konnten.

Als Winterquartiere für *A. maculipennis* konnten hauptsächlich Keller ermittelt werden. An einer Stelle wurde im Freien in einer hohlen Weide *A. messeae* mit *Theobaldia annullata* Schrank in Ueberwinterung gefunden. Die von November bis Februar gefangenen Larven von *A. claviger* befanden sich nicht in echter Winterruhe. *A. plumbeus* bevorzugte Wasser mit einem pH von 6.5 - 7.5. Ende August gesammelte Larven des 4. Stadiums dieser Art wuchsen im Laboratorium raschzu Imagines heran. Larven des 2. Stadiums, die Mitte September eingebracht wurden, erreichten bis Dezember das 3. Stadium. Die Entwicklung zur Imago wurde aber erst zwischen März und Ende April abgeschlossen.

Von besonderem Interesse ist das durch Scherpner festgestellte Vorkommen des mediterranen *A. algeriensis* bei Frankfurt, der bisher in Deutschland erst an 3 Stellen gefunden worden ist: am Niederrhein, im Spreewald und in Mecklenburg. Ein grösserer Larvenbestand von *A. algeriensis* fand sich von 1956 - 1958 in einem Gartenteich südlich von Frankfurt. Im Januar wurde einmal ein überwinterndes Weibchen in einem Gewächshaus unweit des Brutplatzes gefunden. Der Bestand erlosch nach Beseitigung des Brutplatzes.

Untersuchungen über Schwankungen in der Zusammensetzung von Anopheles-Populationen in Norddeutschland.

Eigene Untersuchungen in Norddeutschland sollten die Frage klären, ob gemischte Populationen von *A. maculipennis* über längere Zeiträume hinweg in ihrer Zusammensetzung konstant bleiben oder sich so weit ändern können, dass eine Art die andere verdrängt. Nachteilig ist, dass eine sichere Determination der Anophelen aus der *maculipennis*-Gruppe nur nach den Gelegen erfolgen kann. Eine rasche Bestimmung von im Freien gefangenen Mücken ist also normalerweise nur bei trächtigen Weibchen möglich. Abgesehen davon, dass die Zahl der bestimmbaren Mücken und der Zeitpunkt der Untersuchung dadurch stark beschränkt sind, haben in Gebieten mit gemischten Populationen eine Reihe von Faktoren auf die Zusammensetzung Einfluss und schwächen dadurch den Wert von Einzelbeobachtungen ab. Wir wissen,

dass Physiologie, Ethologie und Oekologie der beiden häufigsten deutschen Arten, *A. atroparvus* und *A. messeae*, sich in wichtigen Punkten unterscheiden. Der Zeitpunkt der Untersuchung und der engere Fundplatz spielen daher für die Auswertung eine grosse Rolle. Die Populationen haben in einem Viehstall eine andere Zusammensetzung als z.B. in einem Schlafraum oder im Freien. Im Stall trift man im Winter nicht die gleichen Arten an wie im Sommer, weil Uberwinterungsmodus und Winterquartiere bei den Arten differieren. Dazu kommen andere Faktoren, deren Bedeutung wir noch nicht in vollem Umfang kennen: Die Sauggewohnheiten, die klimatischen und mikroklimatischen Prädilekta und das örtliche Grossklima, insbesondere Temperatur und Niederschlagshöhe, die auf dem Umweg über die Brutplätze in erster Linie auf die Larven einwirken, aber auch Lebensdauer und Verhalten der Imagines entscheidend beeinflussen können.

Für eine zuverlässige Aussage über die Zusammensetzung und Aederungen der Population in einem bestimmten Gebiet müssten daher an charakteristischen Biotopen Mücken in grösserer Zahl und über einen längeren Zeitraum in kurzen Abständen gefangen und bestimmt werden. Obwohl eine solche Forderung in der Praxis schwer zu verwirklichen ist, haben wir unter diesem Gesichtspunkt an 2 Biotopen in Bauerngehöften in Norddeutschland, der eine in Ostfriesland nördlich von Emden, der andere in den Elbmarschen bei Hamburg gelegen, seit 1931 so oft wie möglich die dort vorkommenden Anophelen nach den Gelegen bestimmt. Ostfriesland war auf Grund der ersten Funde als fast reines *atroparvus*-Gebiet anzusehen, während die Elbmarschen durch eine Mischung von *A. atroparvus* und *A. messeae* besonderes Interesse boten.

Die Ergebnisse der Gelegebestimmungen sind, zeitlich und unter Zusammenfassung der über das ganze Jahr verteilten Einzelfänge geordnet, in Tab 1 wiedergegeben. Wie stark das Fangdatum das Bestimmungsergebnis beeinflussen kann, erkennt man aus Tab. 2. Hier sind die Beobachtungszahlen für den Fundplatz in den Elbmarschen nach der Jahreszeit bzw. nach den Generationen aufgeschlüsselt. Die Mücken der Frühjahrs- und Sommergenerationen befanden sich in voller Fortpflanzungsaktivität. Die Weibchen legten bald nach dem Fangen Eier, während die im Zustand der gonotrophischen Dissoziation befindlichen Mücken aus der Ueberwinterungsphase nur teilweise und erst nach längerer Haltung im Laboratorium bei höherer Temperatur und mehrmaliger Fütterung zur Ablage gebracht werden konnten.

Weitaus die meisten Anophelen wurden in Viehställen gefangen. Die Vollüberwinterung von *A. messeae* und die Semihibernatio von *A. atroparvus* wirken sich dahin aus, dass sich die Mücken, die während der Fortpflanzungsphase die gleichen Tagesbleiben haben, im Herbst trennen. *A. atroparvus* bleibt im Stall oder doch in der Nähe vom Vieh an relativ warmen Plätzen und ergänzt im Winter die verbrauchten Fettreserven durch gelegentliche Blut-

TAB. 2.

Aenderung in der Zusammensetzung der Population in Abhängigkeit vom Jahreszyklus. Biotop in den Elbmarschen. Bestimmung nach den Gelegen.

Datum	Zahl der Gelege (%)	
	A. atroparvus	*A. messeae*
a) *Frühjahrsgeneration nach der Uberwinterung*		
3. 5. 33	27 (29.3)	63 (70.7)
24. 3. 36	15 (37.5)	25 (62.5)
5. 5. 36	16 (28.6)	40 (71.4)
9. 4. 37	16 (69.6)	7 (30.4)
5. 3. 40	167 (72.6)	63 (27.4)
17. 5. 46	9 (30.0)	21 (70.0)
20. 4. 49	30 (50.8)	29 (49.2)
4. 5. 50	26 (72.2)	10 (27.8)
3. 5. 51	15 (48.4)	16 (51.6)
26. 4. 57	8 (14.5)	47 (85.5)
4. 5. 58		12 (100.0)
zusammen	329 (49.6)	333 (50.4)
b) *Sommergeneration*		
20. 8. 31	10 (16.1)	52 (83.9)
28. 7. 32	5 (25.0)	15 (75.0)
21. 7. 36	46 (68.7)	11 (28.2)
10. 6. 37	65 (57.5)	48 (42.5)
20. 7. 49	39 (83.0)	8 (17.0)
5. 7. 57		114 (100.0)
24. 7. 57		153 (100.0)
22. 8. 57	2 (2.2)	87 (97.8)
19. 6. 58	2 (2.5)	77 (97.5)
21. 8. 58	7 (24.1)	22 (75.9)
10. 7. 59	9 (13.6)	66 (86.4)
zusammen	185 (22.1)	653 (77.9)
c) *Uberwinternde Generation*		
Winter 1936/37	28 (71.8)	11 (28.2)
Winter 1937/38	35 (72.9)	13 (27.1)
Winter 1938/39	214 (90.3)	23 (9.7)
10. 2. 39	101 (95.3)	5 (4.7)
11. 10. 39	130 (87.2)	19 (12.8)
18. 11. 43	12 (52.2)	11 (47.8)
28. 8. 55	6 (22.2)	21 (77.8)
2. 9. 59	33 (100.0)	
zusammen	559 (84.4)	103 (15.6)

mahlzeiten. *A. messeae,* der während der winterlichen Diapause kein Blut saugt, zieht sich an kühle Stellen in Kellern, Schuppen, Scheunen, Vorratskammern, auf Dachböden und dergl. zurück. Daher wird man im Winter im Stall eine fast reine *atroparvus*-Population antreffen, während zu derselben Zeit an anderen Plätzen *A. messeae* überwiegt. Dass in den Stallfängen im Winter *A. atroparvus* stärker vertreten ist, zeigt auch Tab. 2. Die Zusammensetzung der Population im Spätsommer und Herbst ebenso wie im zeitigen Frühjahr wird durch den Zeitpunkt der Ein- und Auswinterung bestimmt, der ebenfalls bei beiden Arten differiert. In einem Fang am 5. 3. 1940 (Tab. 2) überwog z. B. *A. atroparvus* nur deshalb, weil ein Teil der *messeae*-Weibchen noch in den Winterquartieren sass, und in einem Fang vom 2. 9. 1949, weil sich die *messeae*-Weibchen nicht mehr im Stall, sondern alle bereits an ihren Ueberwinterungsplätzen auf dem Dachboden befanden.

Auch unter Berücksichtigung der erwähnten Schwierigkeiten und Einschränkungen kann konstatiert werden, dass die *Anopheles*-Population in Ostfriesland im Verlauf einer sich nunmehr auf 27 Jahre erstreckenden Beobachtungszeit konstant geblieben ist (Tab. 1a). Der geringe Anteil von *A. messeae* schwankt in engen Grenzen und erreicht nur ausnahmsweise einmal 16.3%. In den meisten Fällen enthält die Population über 90% *A. atroparvus,* für die gesamte Beobachtungszeit im Durchschnitt 92.1% Auch grössere Klimaschwankungen, sehr warme oder kühle, sehr feuchte oder sehr trockne Sommer, wie sie während dieser Periode mehrfach vorgekommen sind, haben daran nichts geändert.

Etwas anders scheinen auf den ersten Blick die Verhältnisse in dem Gebiet einer Mischpopulation in den Elbmarschen zu liegen (Tab. 1b). Hier schwankt der Anteil der Arten in der Population zwischen 4.0 und 90.0% für *A. atroparvus* und 9.4 und 96.0% für *A. messeae.* In den letzten Jahren schien der *messeae*-Anteil stark zuzunehmen; einige Fänge enthielten nur noch *A. messeae.* Wie Tab. 2 zeigt, beschränkt sich der erhöht *messeae*-Anteil aber auf die Sommergenerationen und kann daher zufällig sein. Im Herbst und Winter erfolgte dann offenbar ein Ausgleich, so dass beide Arten im nächsten Frühjahr wieder annähernd in gleicher Stärke auftraten. Wird das Gleichgewicht gestört - und das scheint während der Beobachtungszeit mehrmals der Fall gewesen zu sein -, so pendelt es sich spätestens im Laufe weniger Jahre wieder ein. Die Populationszusammensetzung ist damit auch hier im wesentlichen die gleiche geblieben.

Diskussion

Die heutige Zusammensetzung der *Anopheles*-Fauna in Deutschland kann als das Ergebnis einer vorwiegend natürlichen Entwicklung angesehen werden, d. h. sie ist unbeeinflusst durch grössere Bodenregulierungen, Aenderungen im

Landschaftscharakter oder gar nachhaltige Bekämpfungsmassnahmen, insbesondere durch den Einsatz von synthetischen Insektiziden. Nachdem in den Kriegs - und Nachkriegsjahren durch eine Vermehrung der Brutplätze, verstärkte Kleintierhaltung, fehlende Gewässerregulierung und mangelnde Wohnungshygiene die Faunendichte vor allem in den nichtlandwirtschaftlichen Gebieten erheblich zugenommen hatte, ist inzwischen wieder eine Normalisierung eingetreten. Untersuchungen über Vorkommen und Lebensweise der Anophelen in Deutschland, die in den ersten Nachkriegsjahren unter dem Eindruck einer beträchtlichen autochthonen Malaria intensiviert wurden, haben unsere Kenntnisse in manchen Punkten erweitert, ohne wesentliche Neuentdeckungen zu bringen. In einzelnen Gebieten, z. B. in Thüringen, am Niederrhein und in Süddeutschland, wurden u. a. Daten über Verbreitung und Häufigkeit der Arten, den Charakter der Brutplätze und die Stechgewohnheiten gesammelt. Für *A. plumbeus* und *A. algeriensis* sind neue Fundplätze ermittelt worden. Vorherrschend sind in Deutschland die Arten aus dem *maculipennis*-Kreis. Für die Beurteilung der Frage, ob die Fauna konstant geblieben ist oder sich geändert hat, ist eine genauere Kenntnis der Lebensgewohnheiten dieser Arten erforderlich.

Die kritische, sich auf einen Zeitraum von über 25 Jahre erstreckende Kontrolle zweier günstiger Biotope in Norddeutschland hat ergeben, dass die Zusammensetzung natürlicher *maculipennis*-Populationen wohl Schwankungen unterliegt, aber grundsätzlich unverändert geblieben ist. In Ostfriesland ist nach wie vor *A. atroparvus* mit über 90% die vorherrschende Art. Es ist daher auch nicht möglich, das Erlöschen der letzten endemischen Malaria in diesem Gebiet auf eine Zunahme von *A. messeae* zurückzuführen. Uberdies hat die Nachkriegsmalaria gezeigt, dass *A. messeae* auch in Deutschland eine autochthone Malaria unterhalten kann.

In einem Gebiet mit einer annähernd gleichmässigen Mischung von *A. messeae* und *A. atroparvus* traten in Abhängigkeit von meteorologischen Einflüssen in den einzelnen Jahren erhebliche Schwankungen der Artanteile auf. Die Unterschiede glichen sich aber gewöhnlich schon in einem Jahr wieder aus. Die Mücken verfügen zum Ueberdauern klimatisch ungünstiger Perioden über ein bestimmtes Anpassungsvermögen, das besonders bei *A. atroparvus* ausgeprägt ist. Diese Eigenschaft teilt die Art offenbar mit *A. labranchiae* Falleroni (Mariani 1957). *A. messeae* wird durch kühle und regenreiche Sommer besonders begünstigt.

Eine allgemeine Tendenz liegt darin, dass die Zahl der Anophelen in Deutschland in den letzten Jahren überall stark abgenommen hat, auch dort, wo sich die Biotope, insbesondere der Charakter der Brutplätze und der Tagesbleiben, nicht erkennbar geändert haben. Warscheinlich hat dieser Rückgang der Faunendichte zum Verschwinden der deutschen Malaria mit beigetragen. Die Gründe für den Abfall der Mückenhäufigkeit, der nicht auf Deutschland

beschränkt ist, sind noch nicht geklärt. Es ist möglich, dass dabei das Sinken des Grundwasserspiegels in Zusammenhang mit der Niederschlagsarmut eine wichtige Rolle spielt.

SULLE VARIAZIONI DELLA COMPOSIZIONE DI POPOLAZIONI NATURALI DI *ANOPHELES* IN GERMANIA

Dopo aver commentato recenti studi sulla ecologia e biologia degli Anofelini in Germania, l'A. riferisce sulle sue ricerche personali.

Per un periodo di 25 anni sono stati notati a regolari intervalli i cambiamenti nella popolazione anofelinica di due biotopi naturali della Germania settentrionale non ancora sottoposti all'uso di insetticidi o ad altre misure di controllo. Sono brevemente discussi i possibili errori risultanti da una valutazione non critica di catture saltuarie.

In uno dei biotopi, situato nella Frisia orientale, la partecipazione di *Anopheles atroparvus* raggiunge il 90%, mentre l'altro biotopo, che si trova nelle paludi del fiume Elba, accoglie una popolazione mista di *A. atroparvus* e *A. messeae*. In media, dall'inizio delle osservazioni, nel 1931, la popolazione non è cambiata; e ciò è particolarmente vero per il biotopo nella Frisia orientale. Per questo, la scomparsa dell'ultima malaria terzana endemica in questa zona non può essere stata causata da un aumento relativo della popolazione di *A. messeae* a spese di una diminuzione di *A. atroparvus*. Nelle paludi dell'Elba d'altra parte la composizione della popolazione ha fluttuato in maggior misura, specie in dipendenza di fattori metereologici. Queste differenze si bilanciarono tuttavia nel corso dello stesso anno od in quello successivo. La densità degli Anofelini è diminuita considerevolmente durante gli ultimi anni senza che sia stata presa alcuna misura degna di menzione.

ON THE VARIATION OF NATURAL ANOPHELES-POPULATION COMPOSITION IN GERMANY

After commenting upon recent studies of the ecology and biology of Anopheline mosquitoes in Germany, the author reports his own investigations.

For a period of more than 25 years changes in the Anopheline population of two natural biotops in Northern Germany, not yet affected by the use of insecticides or other control measures, have been noted at regular intervals. Possible errors resulting from an uncritical evaluation of discontinued catches are briefly discussed.

In one of the biotopes, situated in East Frisia, the share of *Anopheles atroparvus* amounts to 90%, whereas the other biotope, situated in the marshes of the River Elbe, harbours a mixed population of *A. atroparvus* and *A. messeae*. On an average, the composition of the population has not changed since commencing observations in 1931. This is particularly true for the biotope in East Frisia. Hence, the disappearance of the last endemic tertian malaria in this area cannot have been caused by a relative increase of the *A. messeae* population at the expense of a decrease of *A. atroparvus*. In the marshes of the River Elbe, on the other hand, the composition of the population fluctuated more considerably, mainly due to meteorological factors. However, these differences were balanced again either during the same or within the next year. The density of Anophelines has decreased remarkably within the last years without any control measures worth mentioning.

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